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EXHIBIT 2

Ser. No. 09/609,915

Copy of the Papers Filed on April 15, 2002, including a Preliminary Amendment, Replacement computer readable form and Substitute paper copy of Sequence listing

Dkt. 30436.30USI2/SBA/HP

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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant: Peter S. Linsley et al.

Serial No.: 09/609,915 Examiner: E. M. Lazar-Wesley, Ph.D.

Filed: July 3, 2000 Group Art Unit: 1646

For: SOLUBLE CTLA4 MUTANT MOLECULES AND USES THEREOF

35 N. Arroyo Pkwy., Suite 60
Pasadena, California 91103
April 15, 2002

Honorable Assistant Commissioner for Patents
Washington, D.C. 20231

Dear Sir:

COMMUNICATION IN RESPONSE TO A NOTICE TO COMPLY WITH REQUIREMENTS OF 37 CFR 1.821-1.825 FOR PATENT APPLICATIONS CONTAINING NUCLEOTIDE SEQUENCE AND/OR AMINO ACID SEQUENCE DISCLOSURES DATED MARCH 15, 2002

This communication is submitted in response to an Office Communication dated March 15, 2002, to comply with requirements of 37 C.F.R. 1.821 through 1.825 for sequence listing, in connection with the above-identified application. A one (1) month period for reply was set, making April 15, 2002, the deadline for filing a response to the Notice. Accordingly, this Response is being timely filed. A copy of the above-referenced Notice is submitted herein as Exhibit 1.

In response to the Office Communication, the applicants herein submit a sequence listing including an original paper copy, a computer readable copy, and a Declaration under 37 C.F.R. §1.821(f) stating that the computer readable copy of the sequence listing is identical to the paper

copy (Exhibit 2). The sequence listing contains no new matter and is supported by the specification as originally filed. Accordingly, entry of this amendment is respectfully requested.

No additional fee is deemed necessary in connection with the filing of this Communication. However, if any additional fee is necessary, the Patent Office is authorized to charge the additional fee to Deposit Account No. 50-0306.

Respectfully submitted,

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Assistant Commissioner for Patents
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SIR:

PRELIMINARY AMENDMENT

Please amend the subject application as follows.

In the Specification:

In accordance with 37 C.F.R. 1.121(a)(1)(i) and (ii), please replace the paragraph at page 1, lines 5-10, with the following rewritten paragraph:

-- This application is a continuation-in-part of U.S. Serial No. 08/228,208, filed April 15, 1994, now U.S. Patent No. 6,090,914, issued July 18, 2000, which was a continuation-in-part of U.S. Serial No. 08/008, 898, filed January 22, 1993, now U.S Patent No. 5,770,197, issued June 23, 1998, which was a continuation-in part of U.S. Serial No. 07/723,617, filed June 27, 1991, now abandoned; U.S. Serial No. 09/603,825, filed June 26, 2000, which was a continuation-in-part of U.S. Serial No. 09/014,761, filed January 28, 1998, which claims priority of U.S. Serial No. 60/036,594, filed January 31, 1997, now abandoned; and U.S. Serial No. 08/539,436, filed October 5, 1995, now U.S. Patent No. 6,132,992, issued

October 17, 2000, the contents of all of which are hereby incorporated by reference, in their entirety, into the present application. --.

Please replace the paragraph at page 4, lines lines 14-16, beginning "Figure 3:", with the following rewritten paragraph:

-- Figure 3: Shows the nucleotide (SEQ ID NO.: 1) and complete amino acid sequence (SEQ ID NO.: 2) encoding human CTLA4 receptor fused to the oncostatin M signal peptide, and including the newly identified N-linked glycosylation site, as described in Example 1, infra. --.

Please replace the paragraph at page 6, lines 20-25, please replace the paragraph beginning "Figure 7:", with the following rewritten paragraph:

-- Figure 17: Depicts sequencing alignment of CD28 and CTLA4 family members. Sequences of human (H) (SEQ ID NO.: 7), mouse (M) (SEQ ID NO.: 5), rat (R) (SEQ ID NO.: 6), and chicken (Ch) CD28 (SEQ ID NO.: 8) are aligned with human (SEQ ID NO.: 3) and mouse CTLA4 (SEQ ID NO.: 4). The signal peptides are underlined with a dashed line. The transmembrane domains are underlined with a solid line. The CDR-analogous regions are noted. The dark shaded areas highlight complete conservation of residues while the light shaded areas highlight conservative amino acid substitutions in all family members.--.

Please replace the paragraph at page 7, lines 12-13, beginning "Figure 22:", with the following rewritten paragraph:

-- Figure 22: Depicts the nucleotide (SEQ ID NO.: 9) and amino acid sequence of a CTLA4Ig (SEQ ID NO.: 10) having wildtype extracellular domain of CTLA4. --.

Please replace the paragraph at page 7, lines 15-17, beginning "Figure 23:", with the following rewritten paragraph:

-- Figure 23: Depicts the nucleotide (SEQ ID NO.: 11) and amino acid (SEQ ID NO.: 12) sequences of L104EIg starting at methionine at position +1 to aspartic acid at position +124, or alanine at position −1 to aspartic acid at position +124.--.

Please replace the paragraph at page 7, lines 19-21, beginning "Figure 24:", with the following rewritten paragraph:

-- Figure 24: Depicts the nucleotide (SEQ ID NO.: 13) and amino acid sequence (SEQ ID NO.: 14) of L104EA29YIg starting at methionine at position +1 to aspartic acid at position +124, or alanine at position –1 to aspartic acid at position +124.--.

Please replace the paragraph at page 7, lines 23-25, beginning "Figure 25:", with the following rewritten paragraph:

-- Figure 25: Depicts the nucleotide (SEQ ID NO.: 15) and amino acid sequences (SEQ ID NO.: 16) of L104EA29LIg starting at methionine at position +1 to aspartic acid at position +124, or alanine at position –1 to aspartic acid at position +124. --.

Please replace the paragraph at page 7, lines 27-29, beginning "Figure 26:", with the following rewritten paragraph:

-- Figure 26: Depicts the nucleotide (SEQ ID NO.: 17) and amino acid sequences (SEQ ID NO.: 18) of L104EA29TIg starting at methionine at position +1 to aspartic acid at position +124, or alanine at position –1 to aspartic acid at position +124. --.

Please replace the paragraph at page 8, lines 1-3, beginning "Figure 27:", with the following rewritten paragraph:

-- Figure 27: Depicts the nucleotide (SEQ ID NO.: 19) and amino acid sequences (SEQ ID NO.: 20) of L104EA29WIg starting at methionine at position +1 to aspartic acid at position +124, or alanine at position –1 to aspartic acid at position +124.--.

Please replace the paragraph at page 9, lines 22-25, beginning "Figure 37:", with the following rewritten paragraph:

-- Figure 37: Depicts the results of a FACS assay, showing L104EIg and L104ES25RIg bind CHO cells stably transfected with human CD80 or CD86. A) L104EIg and

L104ES25RIg bind to human CD80 CHO-transfected cells; B) L104EIg and L104ES25RIg bind to human CD86 CHO-transfected cells.--.

Please replace the paragraph at page 11 lines 1-8, beginning "One embodiment", with the following rewritten paragraph:

-- One embodiment of a soluble CTLA4 has been deposited with the American Type Culture Collection (ATCC) in Manassas, Maryland, under the provisions of the Budapest Treaty on May 31, 1991 and has been accorded ATCC accession number: 68629. ATCC 68629 is DNA encoding ***CTLA4***Ig. Additionally, the CTLA4Ig-24 CHO cell line has been deposited with the ATCC under the Budapest Treaty on May 31, 1991 and has been accorded accession number ATCC 10762. DNA encoding L104EA29YIg was submitted for deposit on June 19, 2000 with the American Type Culture Collection (ATCC), 10801 University Blvd., Manassas, VA 20110-2209. The DNA encoding L104EA29YIg has been accorded ATCC accession number PTA-2104. --

Please replace the paragraph at page 33, lines 2-21, beginning "Because a signal peptide", with the following rewritten paragraph:

-- Because a signal peptide for CTLA4 was not identified in the CTLA4 gene, the N-terminus of the predicted sequence of CTLA4 was fused to the signal peptide of oncostatin M (Malik et al., <u>Mol. and Cell. Biol.</u> 9:2847 (1989)) in two steps using overlapping oligonucleotides. For the first step, the oligonucleotide, CTCAGTCTGGTCCTTGCACTCCTG TTTCCAAGCATGGCGAGCATGGCAATGCACGTGGCCCAGCC (SEQ ID NO.: 21) (which encoded the C terminal 15 amino acids from the oncostatin M signal peptide fused to the N terminal 7 amino acids of CTLA4) was used as forward primer, and TTTGGGCTCCTGATCAGAATCTGGGCACGGTTG (SEQ ID NO.: 22) (encoding amino acid residues 119-125 of the amino acid sequence encoding CTLA4 receptor and containing a Bcl I restriction enzyme site) as reverse primer. The template for this step was cDNA synthesized from 1 micro g of total RNA from H38 cells (an HTLV II infected T cell leukemic cell line provided by Drs. Salahudin and Gallo, NCI, Bethesda, MD). A portion of

the PCR product from the first step was reamplified, using an overlapping forward primer, encoding the N terminal portion of the oncostatin M signal peptide and containing a Hind III restriction endonuclease site, CTAGCCACTGAAGCTTCACCAATGGGTGTACTGCTCACACAGAGGACGCTGCT CAGTCTGGTCCTTGCACTC (SEQ ID NO.: 23) and the same reverse primer. The product of the PCR reaction was digested with Hind III and Bcl I and ligated together with a Bcl 1/Xba I cleaved cDNA fragment encoding the amino acid sequences corresponding to the hinge, CH2 and CH3 regions of IgCγ1 into the Hind III/Xba I cleaved expression vector, CDM8 or Hind III/Xba I cleaved expression vector pLN. --.

Please replace the paragraph at page 33, lines 23-29, beginning "A schematic map", with the following rewritten paragraph:

-- A schematic map of the resulting CTLA4Ig fusion construct is shown in Figure 1. Sequences displayed in this figure show the junctions between CTLA4 (upper case letters, unshaded regions) and the signal peptide, SP, of oncostatin M (dark shaded regions), and the hinge, H, of IgC(gamma)1 (stippled regions). The amino acid in parentheses was introduced during construction. Asterisks (*) indicate cysteine to serine mutations introduced in the IgCγ hinge region. The immunoglobulin superfamily V-like domain present in CTLA4 is indicated, as are the CH2 and CH3 domains of IgC(gamma)1. --.

Please replace the paragraph at page 34, lines 21-27, beginning "CTLA4Ig", with the following rewritten paragraph:

-- CTLA4Ig was purified by protein A chromatography from serum-free conditioned supernatants (Figure 2). Concentrations of CTLA4Ig were determined assuming an extinction coefficient at 280 nm of 1.6 (experimentally determined by amino acid analysis of a solution of known absorbance). Molecular weight standards (lanes 1 and 3, Figure 2) and samples (1 micro grams) of CTLA4Ig (lanes 2 and 4) were subjected to SDS-PAGE (4-12% acrylamide gradient) under non-reducing conditions (- beta ME, lanes 1 and 2) or

reducing conditions (+ beta ME, lanes 3 and 4) Proteins were visualized by staining with Coomassie Brilliant Blue. --.

Please replace the paragraph at page 35, lines 19-31, beginning "Because of expression of CTLA4 receptor", with the following rewritten paragraph:

-- Because the expression of CTLA4 receptor protein in human lymphoid cells has not been previously reported, it was necessary to locate a source of CTLA4 mRNA. cDNA was reverse transcribed from the total cellular RNA of H38 cells, as noted above, was used for cloning by PCR. For this purpose, the oligonucleotide, GCAATGCACGTGGCCCAGCCTGCTGTGGTAGTG (SEQ ID NO.: 24) (encoding the first 11 amino acids in the predicted coding sequence) was used as a forward primer, and TGATGTAACATGTCTAGATCAATTGATGGGAATAAAATAAGGCTG (SEQ ID NO.: 25) (homologous to the last 8 amino acids in CTLA4 and containing a Xba I site) as reverse primer. The template again was a cDNA synthesized from 1 micro gram RNA from H38 cells. Products of the PCR reaction were cleaved with the restriction endonucleases Nco I and Xba I and the resulting 316 bp product was gel purified. A 340 bp Hind III/Nco I fragment from the CTLAIg fusion described above was also gel-purified, and both restriction fragments were ligated into Hind III/Xba I cleaved CDM8 to form OMCTLA. --.

Please replace the paragraph at page 36, lines 21-25, beginning "Receptor-immunoglobulin C gamma", with the following rewritten paragraph:

-- Receptor-immunoglobulin C gamma (IgCγ) fusion proteins B7Ig and CD28Ig were prepared as described by Linsley et al., in J. Exp. Med. 173:721-730 (1991), incorporated by reference herein. Briefly, DNA encoding amino acid sequences corresponding to the respective receptor protein (e.g. B7) was joined to DNA encoding amino acid sequences corresponding to the hinge, CH2 and CH3 regions of human IgCγ1. This was accomplished as follows. --.

Please replace the paragraph at page 37, lines 6-25, beginning "Plasmid Construction.", with the following rewritten paragraph:

-- Plasmid Construction. Expression plasmids containing cDNA encoding CD28, (as described by Aruffo and Seed, Proc. Natl. Acad. Sci. USA 84:8573 (1987)), were provided by Drs. Aruffo and Seed (Mass General Hospital, Boston, MA). Plasmids containing cDNA encoding CD5, (as described by Aruffo, Cell 61:1303 (1990)), were provided by Dr. Aruffo. Plasmids containing cDNA encoding B7, (as described by Freeman et al., J. Immunol. 143:2714 (1989)), were provided by Dr. Freeman (Dana Farber Cancer Institute, Boston, MA). For initial attempts at expression of soluble forms of CD28 and B7, constructs were made (OMCD28 and OMB7) as described by Linsley et al., J. Exp. Med., supra, in which stop codons were introduced upstream of the transmembrane domains and the native signal peptides were replaced with the signal peptide from oncostatin M (Malik et al., Mol. Cell Biol. 9:2847 (1989)). These were made using synthetic oligonucleotides for reconstruction (OMCD28) or as primers (OMB7) for PCR. OMCD28, is a CD28 cDNA modified for more efficient expression by replacing the signal peptide with the analogous region from oncostatin M. CD28Ig and B7Ig fusion constructs were made in two parts. The 5' portions were made using OMCD28 and OMB7 as templates and the oligonucleotide, CTAGCCACTGAAGCTTCACCATGGGTGTACTGCTCACAC (SEQ ID NO.: 26) (encoding the amino acid sequence corresponding to the oncostatin M signal peptide) as a forward primer, and either TGGCATGGGCTCCTGATCAGGCTTAGAAGGTCCGGGAAA (SEQ ID NO.: 27) or, TTTGGGCTCCTGATCAGGAAAATGCTCTTGCTTGGTTGT (SEQ ID NO.: 28) as reverse primers, respectively. Products of the PCR reactions were cleaved with restriction endonucleases (Hind III and BclI) as sites introduced in the PCR primers and gel purified.--

Please replace the paragraph at page 37, line 27, through page 38, line 9, beginning "The 3' portion of the fusion constructs", with the following rewritten paragraph:

-- The 3' portion of the fusion constructs corresponding to human IgCγ1 sequences was made by a coupled reverse transcriptase (from Avian myeloblastosis virus; Life Sciences

Associates, Bayport, NY)-PCR reaction using RNA from a myeloma cell line producing human-mouse chimeric mAb L6 (provided by Dr. P. Fell and M. Gayle, Bristol-Myers Squibb Company, Pharmaceutical Research Institute, Seattle, WA) as template. The oligonucleotide, AAGCAAGAGCATTTTCCTGATCAGGAGCCCAAATCTTCTGACAAAACTCACA CATCCCCACCGTCCCCAGCACCTGAACTCCTG (SEQ ID NO.: 29) was used as forward primer, and CTTCGACCAGTCTAGAAGCATCCTCGTGCGACCGCGAGAGC (SEQ ID NO.: 30) as reverse primer. Reaction products were cleaved with BclI and XbaI and gel purified. Final constructs were assembled by ligating HindIII/BclI cleaved fragments containing CD28 or B7 sequences together with BclI/XbaI cleaved fragment containing IgCγ1 sequences into HindIII/XbaI cleaved CDM8. Ligation products were transformed into MC1061/p3 E. coli cells and colonies were screened for the appropriate plasmids. Sequences of the resulting constructs were confirmed by DNA sequencing. --.

Please replace the paragraph at page 38, lines 17-24, beginning "CD5Ig was constructed in identical fashion, using", with the following rewritten paragraph:

-- CD5Ig was constructed in identical fashion, using CATTGCACAGTCAAGCTTCCATGCCCATGGGTTCTCTGGCCACCTTG (SEQ ID NO.: 31) as forward primer and ATCCACAGTGCAGTGATCATTTGGATCCTGGCATGTGAC (SEQ ID NO.: 32) as reverse primer. The PCR product was restriction endonuclease digested and ligated with the IgCγ 1 fragment as described above. The resulting construct (CD5Ig) encoded a mature protein having an amino acid sequence containing amino acid residues from position 1 to position 347 of the sequence corresponding to CD5, two amino acids introduced by the construction procedure (amino acids DQ), followed by DNA encoding amino acids corresponding to the IgCγ 1 hinge region. --.

Please replace the paragraph at page 39, lines 21-30, beginning "Immunostaining and FACS^R Analysis.", with the following rewritten paragraph:

-- Immunostaining and FACS^R Analysis. Transfected CHO or COS cells or activated T cells were analyzed by indirect immunostaining. Before staining, CHO cells were removed from their culture vessels by incubation in PBS containing 10 mM EDTA. Cells were first incubated with murine mAbs 9.3 (Hansen et al., Immunogenetics 10:247 (1980)) or BB-1 (Yokochi et al., J. Immunol. 128:823 (1981)), or with Ig fusion proteins (all at 10 micro grams/ml in DMEM containing 10% FCS) for 1-2 h at 4 °C. Cells were then washed, and incubated for an additional 0.5-2h at 4 °C with a FITC-conjugated second step reagent (goat anti-mouse Ig serum for murine mAbs, or goat anti-human Ig Cγ serum for fusion proteins (Tago, Inc., Burlingame, CA)). Fluorescence was analyzed on a FACS IV^R cell sorter (Becton Dickinson and CO., Mountain View, CA) equipped with a four decade logarithmic amplifier. --.

Please replace the paragraph at page 40, line 28, through page 41, line 5, beginning "mAbs.", with the following rewritten paragraph:

-- mAbs. Murine monoclonal antibodies (mAbs) 9.3 (anti-CD28) and G19-4 (anti-CD3), G3-7 (anti-CD7), BB-1 (anti-B7 antigen) and rat mAb 187.1 (anti-mouse kappa chain) have been described previously (Ledbetter et al., Proc. Natl. Acad. Sci. 84:1384-1388 (1987); Ledbetter et al., Blood 75:1531 (1990); Yokochi et al., supra) and were purified from ascites before use. The hybridoma producing mAb OKT8 was obtained from the ATCC, Rockville, MD, and the mAb was also purified from ascites before use. mAb 4G9 (anti-CD19) was provided by Dr. E. Engleman, Stanford University, Palo Alto, CA). Purified human-mouse chimeric mAb L6 (having human Cγ1 Fc portion) was a gift of Dr. P. Fell and M. Gayle (Bristol-Myers Squibb Pharmaceutical Research Institute, Seattle, WA). --.

Please replace the paragraph at page 41, lines 7-15, beginning "Immunostaining and FACS^{R} Analysis.", with the following rewritten paragraph:

-- Immunostaining and FACS^{R} Analysis. Prior to staining, COS or CHO cells were removed from their culture vessels by incubation in PBS containing 10 mM EDTA. Cells were first incubated with mAbs or Ig fusion proteins at 10 micro grams/ml in DMEM containing 10% FBS for 1-2 hr at 4 degrees C. Cells were then washed, and incubated for an additional 0.5-2 hrs at 4 degrees C with FITC-conjugated goat anti-mouse immunoglobulin or with FITC-conjugated goat anti-human IgCγ serum (both from Tago, Burlingame, CA). When binding of both mAbs and Ig fusion proteins were measured in the same experiment, FITC-conjugated anti-mouse and anti-human second step reagents were mixed together before use. Fluorescence on a total of 10,000 cells was then analyzed by FACS^{R}. --.

Please replace the paragraph at page 44, lines 10-17, beginning "Binding of CTLA4Ig on B7 Positive CHO cells. ", with the following rewritten paragraph:

-- Binding of CTLA4Ig on B7 Positive CHO cells. To further characterize the binding of CTLA4Ig and B7, the binding activity of purified CTLA4Ig on B7^{+} CHO cells and on a lymphoblastoid cell line (PM LCL) was measured in the experiment shown in Figure 5. Amplified transfected CHO cell lines and PM LCLs were incubated with medium only (no addition) or an equivalent concentration of human IgCγ1-containing proteins (10 micro grams/ml) of CD5Ig, CD28Ig or CTLA4Ig. Binding was detected by FACS^{R} following addition of FITC-conjugated goat anti-human Ig second step reagents. A total of 10,000 stained cells were analyzed by FACS^{R}. --.

Please replace the paragraph at page 46, lines 14-19, beginning "Primary mixed lymphocyte reaction (MLR)", with the following rewritten paragraph:

-- Primary mixed lymphocyte reaction (MLR) blasts were stimulated with irradiated T51 lymphoblastoid cells (LC) in the absence or presence of concentrations of murine mAb 9.3 Fab fragments, or B7Ig, CD28Ig or CTLA4Ig immunoglobulin Cγ fusion proteins. Cellular

proliferation was measured by [^{3}H]-thymidine incorporation after 4 days and is expressed as the percentage of incorporation by untreated cultures (21,000 cpm). Figure 9 shows the mean of quadruplicate determinations (SEM $\leq$ 10%).--.

Please replace the paragraph at page 48, lines 8-17, beginning "These results demonstrate", with the following rewritten paragraph:

-- These results demonstrate the first expression of a functional protein product of CTLA4 transcripts. CTLA4Ig, a fusion construct containing the extracellular domain of CTLA4 fused to an IgCγ1 domain, forms a disulfide-linked dimer of M_r approximately 50,000 subunits. Because no interchain disulfides would be predicted to form in the Ig portion of this fusion, it seems likely that cysteines from CTLA4 are involved in disulfide bond formation. The analogous CD28Ig fusion protein (Linsley et al, supra, 1991) also contains interchain disulfide linkage(s). These results suggest that CTLA4 receptor, like CD28 (Hansen et al., Immunogenetics 10:247-260 (1980)), exists on the T cell surface as a disulfide linked homodimer. Although CD28 and CTLA4 are highly homologous proteins, they are immunologically distinct, because the anti-CD28 mAb, mAb 9.3, does not recognize CTLA4 (Figures 4 and 5). --

Please replace the paragraph at page 57, lines 20-23, beginning "In addition, two mutants", with the following rewritten paragraph:

-- In addition, two mutants encoding the residues P103A and Y104A (MYPPAY (SEQ ID NO.: 40) and MYPPPA (SEQ ID NO.: 41), respectively) from the CD28Ig 99MYPPPY104 hexapeptide using CD28Ig as a template were also prepared by the same method. --.

Please replace the paragraph at page 57, line 30, through page 58, line 1, beginning "These primers encoded the following sequences:", with the following rewritten paragraph:

-- These primers encoded the following sequences:

CDM8FP:5'-AATACGACTCACTATAGG (SEQ ID NO.: 33)

CDM8RP:5'-CACCACACTGTATTAACC (SEQ ID NO.: 34)

Please replace the paragraph at page 58, line 29, through page 59, line 2, beginning "HS7, HS8, and HS9 constructs", with the following rewritten paragraph:

-- HS7, HS8, and HS9 constructs were prepared by replacing a ~350 base-pair HindIII/HpaI 5' fragment of HS4, HS4-A, and HS4-B, respectively, with the equivalent cDNA fragment similarly digested from HS5 thus introducing the CDR1-like loop of CTLA4 into those hybrids already containing the CTLA4 CDR3-like region. --.

Please replace the paragraph at page 62, lines 29-31, beginning "Several versions of the model", with the following rewritten paragraph:

-- Several versions of the model with modified assignments of some residues to β-strands or loops were tested using 3D-profile analysis (Luthy et al., 1992, Nature 336:83-85) in order to improve the initial alignment of the CTLA4 extracellular region sequence with an IGSF variable fold. --

Please replace the paragraph at page 63, lines 12-15, beginning "Regions of sequence conservation", with the following rewritten paragraph:

-- Regions of sequence conservation are scattered throughout the extracellular domains of these proteins with the most rigorous conservation seen in the hexapeptide MYPPPY (SEQ ID NO.: 35) motif located in the **CDR**3-like loop of both CTLA4 and CD28 (Figure 17). This suggests a probable role for this region in the interaction with a B7 antigen, e.g., B7-1 and B7-2. --.

Please replace the TABLE B at page 73, lines 1-45, beginning "**TABLE B.** Binding of CTLA4 and CD28 monoclonal antibodies", with the following rewritten paragraph:

-- **TABLE B.** Binding of CTLA4 and CD28 monoclonal antibodies to CTLA4Ig and CD28Ig mutant fusion proteins and to CTLA4/CD28Ig hybrid fusion proteins.

	anti-CTLA4 mAbs			anti-CD28 mAb
	7F8	11D4	10A8	9.3
CTLA4Ig MUTANT FUSION PROTEIN				
AYPPPY (SEQ ID NO.: 36)	+++	+++	+++	-
MAPPPY (SEQ ID NO.: 37)	++	+	++	-
MYAPPY (SEQ ID NO.: 38)	+	-	+	-
MYPAPY (SEQ ID NO.: 39)	+++	++++++	+++	-
MYPPAY (SEQ ID NO.: 40)	+++	-	+	-
MYPPPA (SEQ ID NO.: 41)	+++	++	+++	-
AAPPPY (SEQ ID NO.: 42)	+	++	+++	-
CD28Ig MUTANT FUSION PROTEIN				
MYPPAY (SEQ ID NO.: 40)	-	-	-	-
MYPPPA (SEQ ID NO.: 41)	-	-	-	+
CTLA4/CD28Ig HYBRID FUSION PROTEINS				
HS1	-	-	-	-
HS2	-	-	-	+
HS3	-	-	-	-
HS4	-	-	-	+++
HS5	-	-	-	-
HS6	+	-	-	-
HS4-A	-	-	-	++
HS4-B	-	-	-	++
HS7	-	-	-	+++
HS8	-	+	-	+++
HS9	-	+	-	-
HS10	-	-	-	-
HS11	-	-	-	+
HS12	-	-	-	-
HS13	-	-	-	-
HS14	-	-	-	-
CTLA4Ig	+++	+++	+++	-
CD28Ig	-	-	-	+++

Antibody binding was rated from that seen for wild type protein (+++) to above background (+), and no detectable binding (-).

Please replace the paragraph at page 77, lines 12-17, with the following rewritten paragraph:

-- Experimental data were first fit to a model for a single ligand binding to a single receptor (1-site model, i.e., a simple langmuir system, A+B<->AB), and equilibrium association constants ($K_d=[A]\bullet[B]\backslash[AB]$) were calculated from the equation $R=R_{max}\bullet C/(K_d+C)$. Subsequently, data were fit to the simplest two-site model of ligand binding (i.e., to a receptor having two non-interacting independent binding sites as described by the equation $R=R_{max1}\bullet C\backslash(K_{d1}+C)+R_{max2}\bullet C\backslash(K_{d2}+C)$.--.

Please replace the paragraph at page 84, lines 23-28, beginning "From tyrosine +23 to threonine +30,", with the following rewritten paragraph:

-- From tyrosine +23 to threonine +30, a pool of degenerate forward primers were generated having the following sequence:

5' CGA GGC ATC GCT AGC TTT GTG TGT GAG XXK XXK XXK XXK XXK XXK XXK XXK GAG GTC CGG GTG ACA GT 3' (SEQ ID NO.: 43)

Where X = any of the four nucleotides A, T, G, or C and K = either T, G, or C

Each primer contained a Nhe I restriction enzyme cut site. --.

Please replace the paragraph at page 84, lines 30-32, beginning "The reverse primer had the following sequence:", with the following rewritten paragraph:

-- The reverse primer had the following sequence:

5' GGT TGC CGC ACA GAC TTC GGT CAC CTG GCT GTC AGC CTG CCG AAG CAC TGT CAC CCG GA 3' (SEQ ID NO.: 44) --.

Please replace the paragraph at page 86, lines 24-31, beginning "Five mutants were enriched through these 5 rounds of panning.", with the following rewritten paragraph:

-- Five mutants were enriched through these 5 rounds of panning.

Mut 9	F-E-P-K-R-G-V-Q (SEQ ID NO.: 45)
Mut 19	W-D-Q-Y-T-G-Y-G (SEQ ID NO.: 46)
Mut 71	W-D-A-Y-R-N-Q-Q (SEQ ID NO.: 47)

Mut 45	Y-D-H-P-Y-D-G-Q (SEQ ID NO.: 48)
Mut 4	W-D-Q-H-V-S-R-R (SEQ ID NO.: 49)
CTLA4	Y-A-S-P-G-K-A-T (SEQ ID NO.: 50)

REMARKS

The changes to the specification update the priority claimed in the subject application, provide SEQ ID NOs and ATCC accession number, and correct typographical errors in the subject application.

The amendments to specification at page 1, lines 5-10 merely update the status of the priority documents for the subject application. The amended priorities are supported by the executed combined Declaration and Power of Attorney submitted with the subject application. Thus, the above amendments do not introduce any new matter, and accordingly their entry is respectfully requested.

The amendments to specification at pages 4, lines 14-16; page 6, lines, 20-25; page 7, lines 12-13; page 7, lines 15-17; page 7, lines 19-21; page 7, lines, 23-25; page 7, lines 27-29; page 8, lines 1-3; page 33, lines 1-21; page 35, lines 19-31; page 37, lines 6-25; page 38, lines 1-9 and 17-24; page 57, lines 20-22 and 33; page 58, line 1; page 63, lines 12-15; page 73, lines 10-21; page 84, lines 23-28 and 30-32; page 86, lines 24-31 are merely to provide SEQ ID NOs in the Detailed Description. A sequence listing, including a paper copy, a computer readable form and a Declaration pursuant to 37 C.F.R. §1.821(f) are submitted herein as Exhibit 2. The amendments to incorporate SEQ ID NOs. do not introduce any new matter and are supported by the disclosure as originally filed. Accordingly, entry of these amendments is respectfully requested.

The amendments to specification, at page 11, lines 1-8, provides the ATCC accession number for the DNA encoding L104EA29YIg which was deposited with the ATCC under the provision of Budapest treaty and appropriately referenced in the originally filed

application. The amendment to incorporate the ATCC accession number for the deposited DNA does not introduce any new matter, and accordingly the entry of the amendment is respectfully requested.

The amendments to specification, at page 9, lines 22-25 merely deletes "Figure 35" to correct a typographical error. The above amendment does not introduce any new matter, and accordingly the entry of the amendment is respectfully requested.

The amendments to specification at page 33, lines 19 and 27; page 36, lines 21-25; page 37, line 27; page 38, lines 6 and 17-24; page 39, line 28; page 41, lines 4 and 12; page 44, line 14; page 46, line 16; and page 48, line 10, incorporates the symbol --γ-- to correct a typographical error for IgCγ1. The IgCγ1 is a commonly used abbreviation in the art for IgC gamma1. The term "IgC gamma1" is supported by the specification as originally filed (see pages 11, line 14; 27, line 29, 32, line 29). The above amendment is further supported by U.S. Serial No. 08/228,208 (see page 36, line 27, page 40, line 25), to which this application claims priority. Thus, the above amendments do not introduce any new matter, accordingly their entry is respectfully requested.

The amendments to specification at page 34, line 26, merely incorporates -- beta -- to correct a typographical error. The support for the amendment can be found on page 34, line 26 of the specification as originally filed. The above amendment does not introduce any new data, accordingly its entry is respectfully requested.

The amendment to specification at page 58, line 29, corrects a typographical error by incorporating -- ~ --. The above amendment is supported by U.S. Serial No. 08/228,208 (page 64, line 18), to which this application claims priority. The amendment to correct the typographical error at page 58, line 29 does not incorporate any new data, and accordingly, its entry is respectfully requested.

The amendment to specification at page 62, line 29, corrects a typographical error by incorporating the symbol --β-- for beta strands. The above amendment is supported by U.S. Serial No. 08/228,208 (page 68, line 27), to which this application claims priority. The above amendment does not introduce any new matter, and accordingly, its entry is respectfully requested.

The amendment to specification at page 77, line 13, corrects a typographical error by incorporating -- <-> -- . It is commonly known in the art that a 1-site model for a single ligand binding to a single receptor is represented by a simple langmuir system, A+B<->AB. The amendment is further supported by U.S. Serial No. 09/603,825 (page 23, line 10), to which this application claims priority. Thus the above amendment does not introduce any new matter, and accordingly, the entry of the above amendment is respectfully requested.

The changes in the specification do not involve new matter and entry of them is respectfully requested. If a telephone interview would be of assistance in advancing the prosecution of the subject application, applicants' undersigned attorney invites the Examiner to telephone her at the number provided below.

No additional fee is deemed necessary in connection with the filing of this Amendment. If any additional fees are necessary, the Patent Office is authorized to charge any additional fee to Deposit Account No. 50-0306.

Respectfully submitted,

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MARKED-UP VERSION TO SHOW AMENDMENT OF SPECIFICATION

Please amend the specification at page 1, lines 5-10, to read as follows:

-- This application is a continuation-in-part of U.S. Serial No. 08/228,208, filed April 15, 1994, now U.S. Patent No. 6,090,914, issued July 18, 2000, which was a continuation-in-part of U.S. Serial No. 08/008, 898, filed January 22, 1993, now U.S Patent No. 5,770,197, issued June 23, 1998, which was a continuation-in part of U.S. Serial No. 07/723,617, filed June 27, 1991, now abandoned; U.S. Serial No. 09/603,825, filed June 26, 2000, which was a continuation-in-part of U.S. Serial No. 09/014,761, filed January 28, 1998, which claims priority of U.S. Serial No. 60/036,594, filed January 31, 1997, now abandoned; and U.S. Serial No. 08/539,436, filed October 5, 1995, now U.S. Patent No. 6,132,992, issued October 17, 2000, [U.S. Serial No. 08/539,436, filed October 19, 1995, and U.S. Serial No. not yet known, filed June 26, 2000, which is a continuation in part of U.S. Serial No. 09/014,761, filed January 28, 1998, which claims priority of U.S. Serial No. 60/036,549, filed January 28, 1997, now abandoned,] the contents of all of which are hereby incorporated by reference, in their entirety, into the present application. --.

Please amend the specification at page 4, lines 14-16, to read as follows:

-- Figure 3: Shows the nucleotide (SEQ ID NO.: 1) and complete amino acid sequence (SEQ ID NO.: 2) encoding human CTLA4 receptor fused to the oncostatin M signal peptide, and including the newly identified N-linked glycosylation site, as described in Example 1, infra. --.

Please amend the specification at page 6, lines 20-25, to read as follows:

-- Figure 17: Depicts sequencing alignment of CD28 and CTLA4 family members. Sequences of human (H) (SEQ ID NO.: 7), mouse (M) (SEQ ID NO.: 5), rat (R) (SEQ ID NO.: 6), and chicken (Ch) CD28 (SEQ ID NO.: 8) are aligned with human (SEQ ID NO.: 3) and mouse CTLA4 (SEQ ID NO.: 4). The signal peptides are underlined with a dashed line. The transmembrane domains are underlined with a solid line. The CDR-analogous regions

are noted. The dark shaded areas highlight complete conservation of residues while the light shaded areas highlight conservative amino acid substitutions in all family members.--.

Please amend the specification at page 7, lines 11-12, to read as follows:

-- Figure 22: Depicts the nucleotide (SEQ ID NO.: 9) and amino acid sequence of a CTLA4Ig (SEQ ID NO.: 10) having wildtype extracellular domain of CTLA4. --.

Please amend the specification at page 7, lines 15-17, to read as follows:

-- Figure 23: Depicts the nucleotide (SEQ ID NO.: 11) and amino acid (SEQ ID NO.: 12) sequences of L104EIg starting at methionine at position +1 to aspartic acid at position +124, or alanine at position –1 to aspartic acid at position +124.--.

Please amend the specification at page 7, lines 19-21, to read as follows:

-- Figure 24: Depicts the nucleotide (SEQ ID NO.: 13) and amino acid sequence (SEQ ID NO.: 14) of L104EA29YIg starting at methionine at position +1 to aspartic acid at position +124, or alanine at position –1 to aspartic acid at position +124.--.

Please amend the specification at page 7, lines 23-25, to read as follows:

-- Figure 25: Depicts the nucleotide (SEQ ID NO.: 15) and amino acid sequences (SEQ ID NO.: 16) of L104EA29LIg starting at methionine at position +1 to aspartic acid at position +124, or alanine at position –1 to aspartic acid at position +124. --.

Please amend the specification at page 7, lines 27-29, to read as follows:

-- Figure 26: Depicts the nucleotide (SEQ ID NO.: 17) and amino acid sequences (SEQ ID NO.: 18) of L104EA29TIg starting at methionine at position +1 to aspartic acid at position +124, or alanine at position –1 to aspartic acid at position +124. --.

Please amend the specification at page 8, lines 1-3, to read as follows:

-- Figure 27: Depicts the nucleotide (SEQ ID NO.: 19) and amino acid sequences (SEQ ID NO.: 20) of L104EA29WIg starting at methionine at position +1 to aspartic acid at position +124, or alanine at position –1 to aspartic acid at position +124.--.

Please amend the specification at page 8, lines 22-25, to read as follows: Please replace the paragraph at page 9, lines 22-25, with the following rewritten paragraph:

-- Figure 37: [Figure 35] Depicts the results of a FACS assay, showing L104EIg and L104ES25RIg bind CHO cells stably transfected with human CD80 or CD86. A) L104EIg and L104ES25RIg bind to human CD80 CHO-transfected cells; B) L104EIg and L104ES25RIg bind to human CD86 CHO-transfected cells.--.

Please amend the specification at page 11, lines 1-8, to read as follows:

-- One embodiment of a soluble CTLA4 has been deposited with the American Type Culture Collection (ATCC) in Manassas, Maryland, under the provisions of the Budapest Treaty on May 31, 1991 and has been accorded ATCC accession number: 68629. ATCC 68629 is DNA encoding ***CTLA4***Ig. Additionally, the CTLA4Ig-24 CHO cell line has been deposited with the ATCC under the Budapest Treaty on May 31, 1991 and has been accorded accession number ATCC 10762. DNA encoding L104EA29YIg was submitted for deposit on June 19, 2000 with the American Type Culture Collection (ATCC), 10801 University Blvd., Manassas, VA 20110-2209. [The ATCC accession number has not yet been assigned.] The DNA encoding L104EA29YIg has been accorded ATCC accession number PTA-2104. --

Please amend the specification at page 33, lines 2-21, to read as follows:

-- Because a signal peptide for CTLA4 was not identified in the CTLA4 gene, the N-terminus of the predicted sequence of CTLA4 was fused to the signal peptide of oncostatin M (Malik et al., Mol. and Cell. Biol. 9:2847 (1989)) in two steps using overlapping oligonucleotides. For the first step, the oligonucleotide, CTCAGTCTGGTCCTTGCACTCCTGTTCCAAGCATGGCGAGCATGGCAATGCACG

TGGCCCAGCC (SEQ ID NO.: 21) (which encoded the C terminal 15 amino acids from the oncostatin M signal peptide fused to the N terminal 7 amino acids of CTLA4) was used as forward primer, and TTTGGGCTCCTGATCAGAATCTGGGCACGGTTG (SEQ ID NO.: 22) (encoding amino acid residues 119-125 of the amino acid sequence encoding CTLA4 receptor and containing a Bcl I restriction enzyme site) as reverse primer. The template for this step was cDNA synthesized from 1 micro g of total RNA from H38 cells (an HTLV II infected T cell leukemic cell line provided by Drs. Salahudin and Gallo, NCI, Bethesda, MD). A portion of the PCR product from the first step was reamplified, using an overlapping forward primer, encoding the N terminal portion of the oncostatin M signal peptide and containing a Hind III restriction endonuclease site, CTAGCCACTGAAGCTTCACCAATGGGTGTACTGCTCACACAGAGGACGCTGCT CAGTCTGGTCCTTGCACTC (SEQ ID NO.: 23) and the same reverse primer. The product of the PCR reaction was digested with Hind III and Bcl I and ligated together with a Bcl 1/Xba I cleaved cDNA fragment encoding the amino acid sequences corresponding to the hinge, CH2 and CH3 regions of IgCγ1 into the Hind III/Xba I cleaved expression vector, CDM8 or Hind III/Xba I cleaved expression vector pLN. --.

Please amend the specification at page 33, lines 23-29, to read as follows:

-- A schematic map of the resulting CTLA4Ig fusion construct is shown in Figure 1. Sequences displayed in this figure show the junctions between CTLA4 (upper case letters, unshaded regions) and the signal peptide, SP, of oncostatin M (dark shaded regions), and the hinge, H, of IgC(gamma)1 (stippled regions). The amino acid in parentheses was introduced during construction. Asterisks (*) indicate cysteine to serine mutations introduced in the IgCγ hinge region. The immunoglobulin superfamily V-like domain present in CTLA4 is indicated, as are the CH2 and CH3 domains of IgC(gamma)1. --.

Please amend the specification at page 34, lines 21-27, to read as follows:

-- CTLA4Ig was purified by protein A chromatography from serum-free conditioned supernatants (Figure 2). Concentrations of CTLA4Ig were determined assuming an

extinction coefficient at 280 nm of 1.6 (experimentally determined by amino acid analysis of a solution of known absorbance). Molecular weight standards (lanes 1 and 3, Figure 2) and samples (1 micro grams) of CTLA4Ig (lanes 2 and 4) were subjected to SDS-PAGE (4-12% acrylamide gradient) under non-reducing conditions (- beta ME, lanes 1 and 2) or reducing conditions (+ beta ME, lanes 3 and 4) Proteins were visualized by staining with Coomassie Brilliant Blue. --.

Please amend the specification at page 35, lines 19-31, to read as follows:

-- Because the expression of CTLA4 receptor protein in human lymphoid cells has not been previously reported, it was necessary to locate a source of CTLA4 mRNA. cDNA was reverse transcribed from the total cellular RNA of H38 cells, as noted above, was used for cloning by PCR. For this purpose, the oligonucleotide, GCAATGCACGTGGCCCAGCCTGCTGTGGTAGTG (SEQ ID NO.: 24) (encoding the first 11 amino acids in the predicted coding sequence) was used as a forward primer, and TGATGTAACATGTCTAGATCAATTGATGGGAATAAAATAAGGCTG (SEQ ID NO.: 25) (homologous to the last 8 amino acids in CTLA4 and containing a Xba I site) as reverse primer. The template again was a cDNA synthesized from 1 micro gram RNA from H38 cells. Products of the PCR reaction were cleaved with the restriction endonucleases Nco I and Xba I and the resulting 316 bp product was gel purified. A 340 bp Hind III/Nco I fragment from the CTLAIg fusion described above was also gel-purified, and both restriction fragments were ligated into Hind III/Xba I cleaved CDM8 to form OMCTLA. --.

Please amend the specification at page 36, lines 21-25, to read as follows:

-- Receptor-immunoglobulin C gamma (IgCγ) fusion proteins B7Ig and CD28Ig were prepared as described by Linsley et al., in J. Exp. Med. 173:721-730 (1991), incorporated by reference herein. Briefly, DNA encoding amino acid sequences corresponding to the respective receptor protein (e.g. B7) was joined to DNA encoding amino acid sequences

corresponding to the hinge, CH2 and CH3 regions of human IgCγ1. This was accomplished as follows. --.

Please amend the specification at page 37, lines 6-25, to read as follows:

-- Plasmid Construction. Expression plasmids containing cDNA encoding CD28, (as described by Aruffo and Seed, Proc. Natl. Acad. Sci. USA 84:8573 (1987)), were provided by Drs. Aruffo and Seed (Mass General Hospital, Boston, MA). Plasmids containing cDNA encoding CD5, (as described by Aruffo, Cell 61:1303 (1990)), were provided by Dr. Aruffo. Plasmids containing cDNA encoding B7, (as described by Freeman et al., J. Immunol. 143:2714 (1989)), were provided by Dr. Freeman (Dana Farber Cancer Institute, Boston, MA). For initial attempts at expression of soluble forms of CD28 and B7, constructs were made (OMCD28 and OMB7) as described by Linsley et al., J. Exp. Med., supra, in which stop codons were introduced upstream of the transmembrane domains and the native signal peptides were replaced with the signal peptide from oncostatin M (Malik et al., Mol. Cell Biol. 9:2847 (1989)). These were made using synthetic oligonucleotides for reconstruction (OMCD28) or as primers (OMB7) for PCR. OMCD28, is a CD28 cDNA modified for more efficient expression by replacing the signal peptide with the analogous region from oncostatin M. CD28Ig and B7Ig fusion constructs were made in two parts. The 5' portions were made using OMCD28 and OMB7 as templates and the oligonucleotide, CTAGCCACTGAAGCTTCACCATGGGTGTACTGCTCACAC (SEQ ID NO.: 26) (encoding the amino acid sequence corresponding to the oncostatin M signal peptide) as a forward primer, and either TGGCATGGGCTCCTGATCAGGCTTAGAAGGTCCGGGAAA (SEQ ID NO.: 27) or, TTTGGGCTCCTGATCAGGAAAATGCTCTTGCTTGGTTGT (SEQ ID NO.: 28) as reverse primers, respectively. Products of the PCR reactions were cleaved with restriction endonucleases (Hind III and BclI) as sites introduced in the PCR primers and gel purified.--

Please amend the specification at page 37, lines 27, through page 38, line 9, to read as follows:

-- The 3' portion of the fusion constructs corresponding to human IgCg1 sequences was made by a coupled reverse transcriptase (from Avian myeloblastosis virus; Life Sciences Associates, Bayport, NY)-PCR reaction using RNA from a myeloma cell line producing human-mouse chimeric mAb L6 (provided by Dr. P. Fell and M. Gayle, Bristol-Myers Squibb Company, Pharmaceutical Research Institute, Seattle, WA) as template. The oligonucleotide, AAGCAAGAGCATTTTCCTGATCAGGAGCCCAAATCTTCTGACAAAACTCACA CATCCCCACCGTCCCCAGCACCTGAACTCCTG <u>(SEQ ID NO.: 29)</u> was used as forward primer, and CTTCGACCAGTCTAGAAGCATCCTCGTGCGACCGCGAGAGC <u>(SEQ ID NO.: 30)</u> as reverse primer. Reaction products were cleaved with BclI and XbaI and gel purified. Final constructs were assembled by ligating HindIII/BclI cleaved fragments containing CD28 or B7 sequences together with BclI/XbaI cleaved fragment containing IgCγ1 sequences into HindIII/XbaI cleaved CDM8. Ligation products were transformed into MC1061/p3 <u>E. coli</u> cells and colonies were screened for the appropriate plasmids. Sequences of the resulting constructs were confirmed by DNA sequencing. --.

Please amend the specification at page 38, lines 17-24, to read as follows:

-- CD5Ig was constructed in identical fashion, using CATTGCACAGTCAAGCTTCCATGCCCATGGGTTCTCTGGCCACCTTG <u>(SEQ ID NO.: 31)</u> as forward primer and ATCCACAGTGCAGTGATCATTTGGATCCTGGCATGTGAC <u>(SEQ ID NO.: 32)</u> as reverse primer. The PCR product was restriction endonuclease digested and ligated with the IgCγ 1 fragment as described above. The resulting construct (CD5Ig) encoded a mature protein having an amino acid sequence containing amino acid residues from position 1 to position 347 of the sequence corresponding to CD5, two amino acids introduced by the

construction procedure (amino acids DQ), followed by DNA encoding amino acids corresponding to the IgCγ1 hinge region. --.

Please amend the specification at page 39, lines 21-30, to read as follows:

-- Immunostaining and FACS^{R} Analysis. Transfected CHO or COS cells or activated T cells were analyzed by indirect immunostaining. Before staining, CHO cells were removed from their culture vessels by incubation in PBS containing 10 mM EDTA. Cells were first incubated with murine mAbs 9.3 (Hansen et al., Immunogenetics 10:247 (1980)) or BB-1 (Yokochi et al., J. Immunol. 128:823 (1981)), or with Ig fusion proteins (all at 10 micro grams/ml in DMEM containing 10% FCS) for 1-2 h at 4 °C. Cells were then washed, and incubated for an additional 0.5-2h at 4 °C with a FITC-conjugated second step reagent (goat anti-mouse Ig serum for murine mAbs, or goat anti-human Ig Cγ serum for fusion proteins (Tago, Inc., Burlingame, CA)). Fluorescence was analyzed on a FACS IV^{R} cell sorter (Becton Dickinson and CO., Mountain View, CA) equipped with a four decade logarithmic amplifier. --.

Please amend the specification at page 40, line 8, through page 41, line 5, to read as follows:

-- mAbs. Murine monoclonal antibodies (mAbs) 9.3 (anti-CD28) and G19-4 (anti-CD3), G3-7 (anti-CD7), BB-1 (anti-B7 antigen) and rat mAb 187.1 (anti-mouse kappa chain) have been described previously (Ledbetter et al., Proc. Natl. Acad. Sci. 84:1384-1388 (1987); Ledbetter et al., Blood 75:1531 (1990); Yokochi et al., supra) and were purified from ascites before use. The hybridoma producing mAb OKT8 was obtained from the ATCC, Rockville, MD, and the mAb was also purified from ascites before use. mAb 4G9 (anti-CD19) was provided by Dr. E. Engleman, Stanford University, Palo Alto, CA). Purified human-mouse chimeric mAb L6 (having human Cγ1 Fc portion) was a gift of Dr. P. Fell and M. Gayle (Bristol-Myers Squibb Pharmaceutical Research Institute, Seattle, WA). --.

Please amend the specification at page 41, lines 7-15, to read as follows:

-- Immunostaining and FACS^R Analysis. Prior to staining, COS or CHO cells were removed from their culture vessels by incubation in PBS containing 10 mM EDTA. Cells were first incubated with mAbs or Ig fusion proteins at 10 micro grams/ml in DMEM containing 10% FBS for 1-2 hr at 4 degrees C. Cells were then washed, and incubated for an additional 0.5-2 hrs at 4 degrees C with FITC-conjugated goat anti-mouse immunoglobulin or with FITC-conjugated goat anti-human Ig $C\gamma$ serum (both from Tago, Burlingame, CA). When binding of both mAbs and Ig fusion proteins were measured in the same experiment, FITC-conjugated anti-mouse and anti-human second step reagents were mixed together before use. Fluorescence on a total of 10,000 cells was then analyzed by FACS^R. --.

Please amend the specification at page 44, lines 10-17, to read as follows:

-- Binding of CTLA4Ig on B7 Positive CHO cells. To further characterize the binding of CTLA4Ig and B7, the binding activity of purified CTLA4Ig on B7$^+$ CHO cells and on a lymphoblastoid cell line (PM LCL) was measured in the experiment shown in Figure 5. Amplified transfected CHO cell lines and PM LCLs were incubated with medium only (no addition) or an equivalent concentration of human Ig$C\gamma 1$-containing proteins (10 micro grams/ml) of CD5Ig, CD28Ig or CTLA4Ig. Binding was detected by FACS^R following addition of FITC-conjugated goat anti-human Ig second step reagents. A total of 10,000 stained cells were analyzed by FACS^R. --.

Please amend the specification at page 46, lines 14-19, to read as follows:

-- Primary mixed lymphocyte reaction (MLR) blasts were stimulated with irradiated T51 lymphoblastoid cells (LC) in the absence or presence of concentrations of murine mAb 9.3 Fab fragments, or B7Ig, CD28Ig or CTLA4Ig immunoglobulin $C\gamma$ fusion proteins. Cellular proliferation was measured by [^{3}H]-thymidine incorporation after 4 days and is expressed as the percentage of incorporation by untreated cultures (21,000 cpm). Figure 9 shows the mean of quadruplicate determinations (SEM $\leq$ 10%).--.

Please amend the specification at page 48, lines 8-17, to read as follows:

-- These results demonstrate the first expression of a functional protein product of CTLA4 transcripts. CTLA4Ig, a fusion construct containing the extracellular domain of CTLA4 fused to an IgCγ1 domain, forms a disulfide-linked dimer of M_r approximately 50,000 subunits. Because no interchain disulfides would be predicted to form in the Ig portion of this fusion, it seems likely that cysteines from CTLA4 are involved in disulfide bond formation. The analogous CD28Ig fusion protein (Linsley et al, supra, 1991) also contains interchain disulfide linkage(s). These results suggest that CTLA4 receptor, like CD28 (Hansen et al., Immunogenetics 10:247-260 (1980)), exists on the T cell surface as a disulfide linked homodimer. Although CD28 and CTLA4 are highly homologous proteins, they are immunologically distinct, because the anti-CD28 mAb, mAb 9.3, does not recognize CTLA4 (Figures 4 and 5). --

Please amend the specification at page 57, lines 20-23, to read as follows:

-- In addition, two mutants encoding the residues P103A and Y104A (MYPPAY (SEQ ID NO.: 40) and MYPPPA (SEQ ID NO.: 41), respectively) from the CD28Ig 99MYPPPY104 hexapeptide using CD28Ig as a template were also prepared by the same method. --.

Please amend the specification at page 57, lines 30, through page 58, line 1, to read as follows:

-- These primers encoded the following sequences:

CDM8FP:5'-AATACGACTCACTATAGG (SEQ ID NO.: 33)

CDM8RP:5'-CACCACACTGTATTAACC (SEQ ID NO.: 34)

Please amend the specification at page 58, line 29, through page 59, line 2, to read as follows:

-- HS7, HS8, and HS9 constructs were prepared by replacing a ~350 base-pair HindIII/HpaI 5' fragment of HS4, HS4-A, and HS4-B, respectively, with the equivalent cDNA fragment similarly digested from HS5 thus introducing the CDR1-like loop of CTLA4 into those hybrids already containing the CTLA4 CDR3-like region. --.

Please amend the specification at page 62, lines 29-31, to read as follows:

-- Several versions of the model with modified assignments of some residues to β-strands or loops were tested using 3D-profile analysis (Luthy et al., 1992, Nature 336:83-85) in order to improve the initial alignment of the CTLA4 extracellular region sequence with an IGSF variable fold. --

Please amend the specification at page 63, lines 12-15, to read as follows:

-- Regions of sequence conservation are scattered throughout the extracellular domains of these proteins with the most rigorous conservation seen in the hexapeptide MYPPPY (SEQ ID NO.: 35) motif located in the **CDR**3-like loop of both CTLA4 and CD28 (Figure 17). This suggests a probable role for this region in the interaction with a B7 antigen, e.g., B7-1 and B7-2. --.

Please amend the specification at page 73, lines 1-45, to read as follows:

-- **TABLE B.** Binding of CTLA4 and CD28 monoclonal antibodies to CTLA4Ig and CD28Ig mutant fusion proteins and to CTLA4/CD28Ig hybrid fusion proteins.

	anti-CTLA4 mAbs			anti-CD28 mAb
	7F8	11D4	10A8	9.3
CTLA4Ig MUTANT FUSION PROTEIN				
AYPPPY (SEQ ID NO.: 36)	+++	+++	+++	-
MAPPPY (SEQ ID NO.: 37)	++	+	++	-
MYAPPY (SEQ ID NO.: 38)	+	-	+	-
MYPAPY (SEQ ID NO.: 39)	+++	++++++	+++	-
MYPPAY (SEQ ID NO.: 40)	+++	-	+	-

MYPPPA (SEQ ID NO.: 41)	+++	++	+++	-
AAPPPY (SEQ ID NO.: 42)	+	++	+++	-

CD28Ig MUTANT FUSION PROTEIN

MYPPAY (SEQ ID NO.: 40)	-	-	-	-
MYPPPA (SEQ ID NO.: 41)	-	-	-	+

CTLA4/CD28Ig HYBRID FUSION PROTEINS

HS1	-	-	-	-
HS2	-	-	-	+
HS3	-	-	-	-
HS4	-	-	-	+++
HS5	-	-	-	-
HS6	+	-	-	-
HS4-A	-	-	-	++
HS4-B	-	-	-	++
HS7	-	-	-	+++
HS8	-	+	-	+++
HS9	-	+	-	-
HS10	-	-	-	-
HS11	-	-	-	+
HS12	-	-	-	-
HS13	-	-	-	-
HS14	-	-	-	-
CTLA4Ig	+++	+++	+++	-
CD28Ig	-	-	-	+++

Antibody binding was rated from that seen for wild type protein (+++) to above background (+), and no detectable binding (-). --.

Please amend the specification at page 77, lines 12-17, to read as follows:

-- Experimental data were first fit to a model for a single ligand binding to a single receptor (1-site model, i.e., a simple langmuir system, A+B<->AB), and equilibrium association constants ($K_d=[A]\bullet[B]\backslash[AB]$) were calculated from the equation $R=R_{max}\bullet C/(K_d+C)$. Subsequently, data were fit to the simplest two-site model of ligand binding (i.e., to a receptor having two non-interacting independent binding sites as described by the equation $R=R_{max1}\bullet C\backslash(K_{d1}+C)+R_{max2}\bullet C\backslash(K_{d2}+C)$.--.

Please amend the specification at page 84, lines 23-28, to read as follows:

-- From tyrosine +23 to threonine +30, a pool of degenerate forward primers were generated having the following sequence:

5' CGA GGC ATC GCT AGC TTT GTG TGT GAG XXK XXK XXK XXK XXK XXK XXK XXK GAG GTC CGG GTG ACA GT 3' <u>(SEQ ID NO.: 43)</u>

Where X = any of the four nucleotides A, T, G, or C and K = either T, G, or C

Each primer contained a Nhe I restriction enzyme cut site. --.

Please amend the specification at page 84, lines 30-32, to read as follows:

-- The reverse primer had the following sequence:

5' GGT TGC CGC ACA GAC TTC GGT CAC CTG GCT GTC AGC CTG CCG AAG CAC TGT CAC CCG GA 3' <u>(SEQ ID NO.: 44)</u> --.

Please amend the specification at page 86, lines 24-31, to read as follows:

-- <u>Five mutants were enriched through these 5 rounds of panning.</u>

Mut 9	F-E-P-K-R-G-V-Q <u>(SEQ ID NO.: 45)</u>
Mut 19	W-D-Q-Y-T-G-Y-G <u>(SEQ ID NO.: 46)</u>
Mut 71	W-D-A-Y-R-N-Q-Q <u>(SEQ ID NO.: 47)</u>
Mut 45	Y-D-H-P-Y-D-G-Q <u>(SEQ ID NO.: 48)</u>
Mut 4	W-D-Q-H-V-S-R-R <u>(SEQ ID NO.: 49)</u>
CTLA4	Y-A-S-P-G-K-A-T <u>(SEQ ID NO.: 50)</u>

EXHIBIT 1

Copy of Notice to Comply with Requirements for Patent Applications Containing Nucleotide Sequence and/or Amino Acid Sequence Disclosures

NOTICE TO COMPLY WI REQUIREMENTS FOR PATENT A ICATIONS CONTAINING NUCLEOTIDE SEQUENCE AND/OR AMINO ACID SEQUENCE DISCLOSURES

The nucleotide and/or amino acid sequence disclosure contained in this application does not comply with the requirements for such a disclosure as set forth in 37 CFR 1.821 - 1.825 for the following reason(s):

☑ 1. This application clearly fails to comply with the requirements of 37 CFR 1.821 - 1.825. Applicant's attention is directed to these regulations, published at 1114 OG 29, May 15, 1990 and at 55 FR 18230, May 1, 1990.

☑ 2. This application does not contain, as a separate part of the disclosure on paper copy, a "Sequence Listing" as required by 37 CFR 1.821(c).

☐ 3. A copy of the "Sequence Listing" in computer readable form has not been submitted as required by 37 CFR 1.821(e).

☐ 4. A copy of the "Sequence Listing" in computer readable form has been submitted. However, the content of the computer readable form does not comply with the requirements of 37 CFR 1.822 and/or 1.823, as indicated on the attached copy of the marked-up "Raw Sequence Listing."

☐ 5. The computer readable form that has been filed with this application has been found to be damaged and/or unreadable as indicated on the attached CRF Diskette Problem Report. A substitute computer readable form must be submitted as required by 37 CFR 1.825(d).

☐ 6. The paper copy of the "Sequence Listing" is not the same as the computer readable form of the "Sequence Listing" as required by 37 CFR 1.821(e).

☒ 7. Applicants should follow the format of the attached sample

Other: statement if they request that the CRF filed in the parent

Applicant must provide: application should be used to create a CRF in this application.

☑ An initial or substitute computer readable form (CRF) copy of the "Sequence Listing"

☑ An initial or substitute paper copy of the "Sequence Listing", as well as an amendment directing its entry into the specification

☑ A statement that the content of the paper and computer readable copies are the same and, where applicable, include no new matter, as required by 37 CFR 1.821(e) or 1.821(f) or 1.821(g) or 1.825(b) or 1.825(d)

For questions regarding compliance with these requirements, please contact:

For Rules Interpretation, call (703) 308-1123
For CRF submission help, call (703) 308-4212
For PatentIn software help, call (703) 557-0400

Please return a copy of this notice with your response.

EXHIBIT 2

Sequence Listing in paper copy and Declaration Pursuant to 37 C.F.R. §1.821(f)

SEQUENCE LISTING

<110> Linsley, Peter S
Ledbetter, Jeffrey A
Bajorath, Jurgen
Peach, Robert J
Brady, William
Wallace, Philip
Damle, Nitin K

<120> SOLUBLE CTLA4 MUTANT MOLECULES AND USES THEREOF

<130> 30436.30USI2

<140> 09/609,915
<141> 2000-07-03

<150> 07/723,617
<151> 1991-06-27

<150> 08/008,898
<151> 1993-01-22

<150> 08/228,208
<151> 1994-04-15

<150> 08/539,436
<151> 1995-10-05

<150> 09/014,761
<151> 1998-01-28

<150> 09/603,825
<151> 2000-06-26

<150> 60/036,594
<151> 1997-01-31

<160> 50

<170> PatentIn version 3.1

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<213> Homo sapiens

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ggcatcgcca gctttgtgtg tgagtatgca tctccaggca aagccactga ggtccgggtg 180

acagtgcttc ggcaggctga cagccaggtg actgaagtct gtgcggcaac ctacatgatg 240

gggaatgagt tgaccttcct agatgattcc atctgcacgg gcacctccag tggaaatcaa 300

gtgaacctca ctatccaagg actgagggcc atggacacgg gactctacat ctgcaaggtg 360

gagctcatgt acccaccgcc atactacctg ggcataggca acggaaccca gatttatgta 420

attgatccag aaccgtgccc agattctgac ttcctcctct ggatccttgc agcagttagt 480

tcggggttgt ttttttatag ctttctcctc acagctgttt ctttgagcaa aatgctaaag 540

aaaagaagcc ctcttacaac aggggtctat gtgaaaatgc cccaacaga gccagaatgt 600

gaaaagcaat ttcagcctta ttttattccc atcaat 636

<210> 2
<211> 212
<212> PRT
<213> Homo sapiens

<400> 2

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Met Gly Val Leu Leu Thr Gln Arg Thr Leu Leu Ser Leu Val Leu Ala
1               5                   10                  15

Leu Leu Phe Pro Ser Met Ala Ser Met Ala Met His Val Ala Gln Pro
            20                  25                  30

Ala Val Val Leu Ala Ser Ser Arg Gly Ile Ala Ser Phe Val Cys Glu
        35                  40                  45

Tyr Ala Ser Pro Gly Lys Ala Thr Glu Val Arg Val Thr Val Leu Arg
    50                  55                  60

Gln Ala Asp Ser Gln Val Thr Glu Val Cys Ala Ala Thr Tyr Met Met
65                  70                  75                  80

Gly Asn Glu Leu Thr Phe Leu Asp Asp Ser Ile Cys Thr Gly Thr Ser
                85                  90                  95

Ser Gly Asn Gln Val Asn Leu Thr Ile Gln Gly Leu Arg Ala Met Asp
            100                 105                 110

Thr Gly Leu Tyr Ile Cys Lys Val Glu Leu Met Tyr Pro Pro Pro Tyr
        115                 120                 125

Tyr Leu Gly Ile Gly Asn Gly Thr Gln Ile Tyr Val Ile Asp Pro Glu
    130                 135                 140
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Pro Cys Pro Asp Ser Asp Phe Leu Leu Trp Ile Leu Ala Ala Val Ser
145 150 155 160

Ser Gly Leu Phe Phe Tyr Ser Phe Leu Leu Thr Ala Val Ser Leu Ser
165 170 175

Lys Met Leu Lys Lys Arg Ser Pro Leu Thr Thr Gly Val Tyr Val Lys
180 185 190

Met Pro Pro Thr Glu Pro Glu Cys Glu Lys Gln Phe Gln Pro Tyr Phe
195 200 205

Ile Pro Ile Asn
210

<210> 3
<211> 223
<212> PRT
<213> Homo sapiens

<400> 3

Met Ala Cys Leu Gly Phe Gln Arg His Lys Ala Gln Leu Asn Leu Ala
1 5 10 15

Ala Arg Thr Trp Pro Cys Thr Leu Leu Phe Phe Leu Leu Phe Ile Pro
20 25 30

Val Phe Cys Lys Ala Met His Val Ala Gln Pro Ala Val Val Leu Ala
35 40 45

Ser Ser Arg Gly Ile Ala Ser Phe Val Cys Glu Tyr Ala Ser Pro Gly
50 55 60

Lys Ala Thr Glu Val Arg Val Thr Val Leu Arg Gln Ala Asp Ser Gln
65 70 75 80

Val Thr Glu Val Cys Ala Ala Thr Tyr Met Met Gly Asn Glu Leu Thr
85 90 95

Phe Leu Asp Asp Ser Ile Cys Thr Gly Thr Ser Ser Gly Asn Gln Val
100 105 110

Asn Leu Thr Ile Gln Gly Leu Arg Ala Met Asp Thr Gly Leu Tyr Ile
115 120 125

```
Cys Lys Val Glu Leu Met Tyr Pro Pro Pro Tyr Tyr Leu Gly Ile Gly
    130                 135                 140

Asn Gly Thr Gln Ile Tyr Val Ile Asp Pro Glu Pro Cys Pro Asp Ser
145                 150                 155                 160

Asp Phe Leu Leu Trp Ile Leu Ala Ala Val Ser Ser Gly Leu Phe Phe
                165                 170                 175

Tyr Ser Phe Leu Leu Thr Ala Val Ser Leu Ser Lys Met Leu Lys Lys
            180                 185                 190

Arg Ser Pro Leu Thr Thr Gly Val Tyr Val Lys Met Pro Pro Thr Glu
        195                 200                 205

Pro Glu Cys Glu Lys Gln Phe Gln Pro Tyr Phe Ile Pro Ile Asn
    210                 215                 220
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<210> 4
<211> 223
<212> PRT
<213> Mus musculus

<400> 4

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Ser Arg Thr Trp Pro Phe Val Ala Leu Leu Thr Leu Leu Phe Ile Pro
            20                  25                  30

Val Phe Ser Glu Ala Ile Gln Val Thr Gln Pro Ser Val Tyr Leu Ala
        35                  40                  45

Ser Ser His Gly Tyr Ala Ser Phe Pro Cys Glu Tyr Ser Pro Ser His
    50                  55                  60

Asn Thr Asp Glu Val Arg Val Thr Val Leu Arg Gln Thr Asn Asp Gln
65                  70                  75                  80

Met Thr Glu Val Cys Ala Thr Thr Phe Thr Glu Lys Asn Thr Val Gly
                85                  90                  95
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Phe Leu Asp Tyr Pro Phe Cys Ser Gly Thr Phe Asn Glu Ser Arg Val
100 105 110

Asn Leu Thr Ile Gln Gly Leu Arg Ala Val Asp Thr Gly Leu Tyr Leu
115 120 125

Cys Lys Val Glu Leu Met Tyr Pro Pro Pro Tyr Phe Val Gly Met Gly
130 135 140

Asn Gly Thr Gln Ile Tyr Tyr Ile Asp Pro Glu Pro Cys Pro Asp Ser
145 150 155 160

Asp Phe Leu Leu Trp Ile Leu Tyr Ala Val Ser Leu Gly Leu Phe Phe
165 170 175

Tyr Ser Phe Leu Val Ser Ala Val Ser Leu Ser Lys Met Leu Lys Lys
180 185 190

Arg Ser Pro Leu Thr Thr Gly Val Tyr Val Lys Met Pro Pro Thr Glu
195 200 205

Pro Glu Cys Glu Lys Gln Phe Gln Pro Tyr Phe Ile Pro Ile Asn
210 215 220

<210> 5
<211> 218
<212> PRT
<213> Mus musculus

<400> 5

Met Thr Leu Arg Leu Leu Phe Leu Ala Leu Asn Phe Phe Ser Val Gln
1 5 10 15

Val Thr Glu Asn Lys Ile Leu Val Lys Gln Ser Pro Leu Leu Tyr Val
20 25 30

Asp Ser Asn Glu Val Ser Leu Ser Cys Arg Tyr Ser Tyr Asn Leu Leu
35 40 45

Ala Lys Glu Phe Arg Ala Ser Leu Tyr Lys Gly Val Asn Ser Asp Val
50 55 60

Glu Val Cys Val Gly Asn Gly Asn Phe Thr Tyr Gln Pro Gln Phe Arg
65 70 75 80

Ser Asn Ala Glu Phe Asn Cys Asp Gly Asp Phe Asp Asn Glu Thr Val
                85   90   95

Thr Phe Arg Leu Trp Asn Leu His Val Asn His Thr Asp Ile Tyr Phe
            100   105   110

Cys Lys Ile Glu Phe Met Tyr Pro Pro Pro Tyr Leu Asp Asn Glu Arg
        115   120   125

Ser Asn Gly Thr Ile Ile His Ile Lys Glu Lys His Leu Cys His Thr
    130   135   140

Gln Ser Ser Pro Lys Leu Phe Trp Ala Leu Tyr Val Val Ala Gly Val
145   150   155   160

Leu Phe Cys Tyr Gly Leu Leu Val Thr Val Ala Leu Cys Val Ile Trp
                165   170   175

Thr Asn Ser Arg Arg Asn Arg Leu Leu Gln Val Thr Tyr Met Asn Met
            180   185   190

Thr Pro Arg Arg Pro Gly Leu Thr Arg Lys Pro Tyr Gln Pro Tyr Ala
        195   200   205

Pro Ala Arg Asp Phe Ala Ala Tyr Arg Pro
    210   215

<210> 6
<211> 218
<212> PRT
<213> Rattus norvegicus

<400> 6

Met Thr Leu Arg Leu Leu Phe Leu Ala Leu Ser Phe Phe Ser Val Gln
1   5   10   15

Val Thr Glu Asn Lys Ile Leu Val Lys Gln Ser Pro Leu Leu Val Tyr
            20   25   30

Asp Asn Asn Glu Val Ser Leu Ser Cys Arg Tyr Ser Tyr Asn Leu Leu
        35   40   45

Ala Lys Glu Phe Arg Ala Ser Leu Tyr Lys Gly Val Asn Ser Asp Val
    50   55   60

Glu Val Cys Val Gly Asn Gly Asn Phe Thr Tyr Gln Pro Gln Phe Arg
65   70   75   80

Pro Asn Val Gly Phe Asn Cys Asp Gly Asn Phe Asp Asn Glu Thr Val
                85   90   95

Thr Phe Arg Leu Trp Asn Leu Asp Val Asn His Thr Asp Ile Tyr Phe
            100   105   110

Cys Lys Ile Glu Val Met Tyr Pro Pro Pro Tyr Leu Asp Asn Glu Lys
        115   120   125

Ser Asn Gly Thr Ile Ile His Ile Lys Glu Lys His Leu Cys His Ala
    130   135   140

Gln Thr Ser Pro Lys Leu Phe Trp Pro Leu Val Val Val Ala Gly Val
145   150   155   160

Leu Leu Cys Tyr Gly Leu Leu Tyr Thr Val Thr Leu Cys Ile Ile Trp
                165   170   175

Thr Asn Ser Arg Arg Asn Arg Leu Leu Gln Ser Asp Tyr Met Asn Met
            180   185   190

Thr Pro Arg Arg Leu Gly Pro Thr Arg Lys His Tyr Gln Pro Tyr Ala
        195   200   205

Pro Ala Arg Asp Phe Ala Ala Tyr Arg Pro
    210   215

<210> 7
<211> 220
<212> PRT
<213> Homo sapiens

<400> 7

Met Leu Arg Leu Leu Leu Ala Leu Asn Leu Phe Pro Ser Ile Gln Val
1   5   10   15

Thr Gly Asn Lys Ile Leu Val Lys Gln Ser Pro Met Leu Val Ala Tyr
            20   25   30

```
Asp Asn Ala Tyr Asn Leu Ser Cys Lys Tyr Ser Tyr Asn Leu Phe Ser
        35                  40                  45

Arg Glu Phe Arg Ala Ser Leu His Lys Gly Leu Asp Ser Ala Val Glu
    50                  55                  60

Val Cys Val Val Tyr Gly Asn Tyr Ser Gln Gln Leu Gln Val Tyr Ser
65                  70                  75                  80

Lys Thr Gly Phe Asn Cys Asp Gly Lys Leu Gly Asn Glu Ser Val Thr
                85                  90                  95

Phe Tyr Leu Gln Asn Leu Tyr Val Asn Gln Thr Asp Ile Tyr Phe Cys
            100                 105                 110

Lys Ile Glu Val Met Tyr Pro Pro Pro Tyr Leu Asp Asn Glu Lys Ser
        115                 120                 125

Asn Gly Thr Ile Ile His Val Lys Gly Lys His Leu Cys Pro Ser Pro
    130                 135                 140

Leu Phe Pro Gly Pro Ser Lys Pro Phe Trp Val Leu Val Val Val Gly
145                 150                 155                 160

Gly Val Leu Ala Cys Tyr Ser Leu Leu Tyr Thr Val Ala Phe Ile Ile
                165                 170                 175

Phe Trp Val Arg Ser Lys Arg Ser Arg Leu Leu His Ser Asp Tyr Met
            180                 185                 190

Asn Met Thr Pro Arg Arg Pro Gly Pro Thr Arg Lys His Tyr Gln Pro
        195                 200                 205

Tyr Ala Pro Pro Arg Asp Phe Ala Ala Tyr Arg Ser
    210                 215                 220
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<210>  8
<211>  221
<212>  PRT
<213>  Gallus gallus

<400>  8
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Met Leu Gly Ile Leu Val Val Leu Cys Leu Ile Pro Ala Ala Asp Val
1               5                   10                  15

Thr Glu Asn Lys Ile Leu Val Ala Gln Arg Pro Leu Leu Ile Val Ala
            20                  25                  30

Asn Arg Thr Ala Thr Leu Val Cys Asn Tyr Thr Tyr Asn Gly Thr Gly
        35                  40                  45

Lys Glu Phe Arg Ala Ser Leu His Lys Gly Thr Asp Ser Ala Val Glu
    50                  55                  60

Val Cys Phe Ile Ser Trp Asn Met Thr Lys Ile Asn Ser Asn Ser Asn
65                  70                  75                  80

Lys Glu Phe Asn Cys Arg Gly Ile His Asp Lys Asp Lys Val Ile Phe
                85                  90                  95

Asn Leu Trp Asn Met Ser Ala Ser Gln Thr Asp Ile Tyr Phe Cys Lys
            100                 105                 110

Ile Glu Ala Met Tyr Pro Pro Pro Tyr Val Tyr Asn Glu Lys Ser Asn
        115                 120                 125

Gly Thr Val Ile His Tyr Arg Glu Thr Pro Ile Gln Thr Gln Glu Pro
    130                 135                 140

Glu Ser Ala Thr Ser Tyr Trp Val Met Tyr Ala Val Thr Gly Leu Leu
145                 150                 155                 160

Gly Phe Tyr Ser Met Leu Ile Thr Ala Val Phe Ile Ile Tyr Arg Gln
                165                 170                 175

Lys Ser Lys Arg Asn Arg Tyr Arg Gln Ser Asp Tyr Met Asn Met Thr
            180                 185                 190

Pro Arg His Pro Pro His Gln Lys Asn Lys Gly Tyr Pro Ser Tyr Ala
        195                 200                 205

Pro Thr Arg Asp Tyr Thr Ala Tyr Arg Ser Trp Gln Pro
    210                 215                 220
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<210> 9

<211> 1152
<212> DNA
<213> Artificial Sequence

<220>
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ggcatcgcta gctttgtgtg tgagtatgca tctccaggca aagccactga ggtccgggtg 180

acagtgcttc ggcaggctga cagccaggtg actgaagtct gtgcggcaac ctacatgatg 240

gggaatgagt tgaccttcct agatgattcc atctgcacgg gcacctccag tggaaatcaa 300

gtgaacctca ctatccaagg actgagggcc atggacacgg gactctacat ctgcaaggtg 360

gagctcatgt acccaccgcc atactacctg ggcataggca acggaaccca gatttatgta 420

attgatccag aaccgtgccc agattctgat caggagccca aatcttctga caaaactcac 480

acatccccac cgtccccagc acctgaactc ctgggtggat cgtcagtctt cctcttcccc 540

ccaaaaccca aggacaccct catgatctcc cggacccctg aggtcacatg cgtggtggtg 600

gacgtgagcc acgaagaccc tgaggtcaag ttcaactggt acgtggacgg cgtggaggtg 660

cataatgcca agacaaagcc gcgggaggag cagtacaaca gcacgtaccg ggtggtcagc 720

gtcctcaccg tcctgcacca ggactggctg aatggcaagg agtacaagtg caaggtctcc 780

aacaaagccc tcccagcccc catcgagaaa accatctcca aagccaaagg gcagccccga 840

gaaccacagg tgtacaccct gcccccatcc cgggatgagc tgaccaagaa ccaggtcagc 900

ctgacctgcc tggtcaaagg cttctatccc agcgacatcg ccgtggagtg ggagagcaat 960

gggcagccgg agaacaacta caagaccacg cctcccgtgc tggactccga cggctccttc 1020

ttcctctaca gcaagctcac cgtggacaag agcaggtggc agcaggggaa cgtcttctca 1080

tgctccgtga tgcatgaggc tctgcacaac cactacacgc agaagagcct ctccctgtct 1140

ccgggtaaat ga 1152

<210> 10
<211> 383
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: CTLA4Ig

<400> 10

Met Gly Val Leu Leu Thr Gln Arg Thr Leu Leu Ser Leu Val Leu Ala
1   5   10   15

Leu Leu Phe Pro Ser Met Ala Ser Met Ala Met His Val Ala Gln Pro
            20   25   30

Ala Val Val Leu Ala Ser Ser Arg Gly Ile Ala Ser Phe Val Cys Glu
        35   40   45

Tyr Ala Ser Pro Gly Lys Ala Thr Glu Val Arg Val Thr Val Leu Arg
    50   55   60

Gln Ala Asp Ser Gln Val Thr Glu Val Cys Ala Ala Thr Tyr Met Met
65   70   75   80

Gly Asn Glu Leu Thr Phe Leu Asp Asp Ser Ile Cys Thr Gly Thr Ser
                85   90   95

Ser Gly Asn Gln Val Asn Leu Thr Ile Gln Gly Leu Arg Ala Met Asp
            100   105   110

Thr Gly Leu Tyr Ile Cys Lys Val Glu Leu Met Tyr Pro Pro Pro Tyr
        115   120   125

Tyr Leu Gly Ile Gly Asn Gly Thr Gln Ile Tyr Val Ile Asp Pro Glu
    130   135   140

Pro Cys Pro Asp Ser Asp Gln Glu Pro Lys Ser Ser Asp Lys Thr His
145   150   155   160

Thr Ser Pro Pro Ser Pro Ala Pro Glu Leu Leu Gly Gly Ser Ser Val
                165   170   175

Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr
            180   185   190

Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu
        195   200   205

Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys
    210   215   220

Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser
225 230 235 240

Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys
245 250 255

Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile
260 265 270

Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro
275 280 285

Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Thr Cys Leu
290 295 300

Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn
305 310 315 320

Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser
325 330 335

Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg
340 345 350

Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu
355 360 365

His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
370 375 380

<210> 11
<211> 1152
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: L104EIg

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ggcatcgcta gctttgtgtg tgagtatgca tctccaggca aagccactga ggtccgggtg 180

acagtgcttc ggcaggctga cagccaggtg actgaagtct gtgcggcaac ctacatgatg 240

gggaatgagt tgaccttcct agatgattcc atctgcacgg gcacctccag tggaaatcaa 300

gtgaacctca ctatccaagg actgagggcc atggacacgg gactctacat ctgcaaggtg 360

gagctcatgt acccaccgcc atactacgag ggcataggca acggaaccca gatttatgta 420

attgatccag aaccgtgccc agattctgat caggagccca aatcttctga caaaactcac 480

acatccccac cgtccccagc acctgaactc ctggggggat cgtcagtctt cctcttcccc 540

ccaaaaccca aggacaccct catgatctcc cggacccctg aggtcacatg cgtggtggtg 600

gacgtgagcc acgaagaccc tgaggtcaag ttcaactggt acgtggacgg cgtggaggtg 660

cataatgcca agacaaagcc gcgggaggag cagtacaaca gcacgtaccg tgtggtcagc 720

gtcctcaccg tcctgcacca ggactggctg aatggcaagg agtacaagtg caaggtctcc 780

aacaaagccc tcccagcccc catcgagaaa accatctcca aagccaaagg gcagccccga 840

gaaccacagg tgtacaccct gcccccatcc cgggatgagc tgaccaagaa ccaggtcagc 900

ctgacctgcc tggtcaaagg cttctatccc agcgacatcg ccgtggagtg ggagagcaat 960

gggcagccgg agaacaacta caagaccacg cctcccgtgc tggactccga cggctccttc 1020

ttcctctaca gcaagctcac cgtggacaag agcaggtggc agcaggggaa cgtcttctca 1080

tgctccgtga tgcatgaggc tctgcacaac cactacacgc agaagagcct ctccctgtct 1140

ccgggtaaat ga 1152

<210> 12
<211> 383
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: L104EIg

<400> 12

Met Gly Val Leu Leu Thr Gln Arg Thr Leu Leu Ser Leu Val Leu Ala
1 5 10 15

Leu Leu Phe Pro Ser Met Ala Ser Met Ala Met His Val Ala Gln Pro
20 25 30

Ala Val Val Leu Ala Ser Ser Arg Gly Ile Ala Ser Phe Val Cys Glu
35 40 45

Tyr Ala Ser Pro Gly Lys Ala Thr Glu Val Arg Val Thr Val Leu Arg

50 55 60

Gln Ala Asp Ser Gln Val Thr Glu Val Cys Ala Ala Thr Tyr Met Met
65 70 75 80

Gly Asn Glu Leu Thr Phe Leu Asp Asp Ser Ile Cys Thr Gly Thr Ser
85 90 95

Ser Gly Asn Gln Val Asn Leu Thr Ile Gln Gly Leu Arg Ala Met Asp
100 105 110

Thr Gly Leu Tyr Ile Cys Lys Val Glu Leu Met Tyr Pro Pro Pro Tyr
115 120 125

Tyr Glu Gly Ile Gly Asn Gly Thr Gln Ile Tyr Val Ile Asp Pro Glu
130 135 140

Pro Cys Pro Asp Ser Asp Gln Glu Pro Lys Ser Ser Asp Lys Thr His
145 150 155 160

Thr Ser Pro Pro Ser Pro Ala Pro Glu Leu Leu Gly Gly Ser Ser Val
165 170 175

Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr
180 185 190

Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu
195 200 205

Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys
210 215 220

Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser
225 230 235 240

Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys
245 250 255

Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile
260 265 270

Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro
275 280 285

Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Thr Cys Leu
290 295 300

Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn
305 310 315 320

Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser
325 330 335

Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg
340 345 350

Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu
355 360 365

His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
370 375 380

<210> 13
<211> 1152
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: L104EA29YIg

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ggcatcgcta gctttgtgtg tgagtatgca tctccaggca aatatactga ggtccgggtg 180

acagtgcttc ggcaggctga cagccaggtg actgaagtct gtgcggcaac ctacatgatg 240

gggaatgagt tgaccttcct agatgattcc atctgcacgg gcacctccag tggaaatcaa 300

gtgaacctca ctatccaagg actgagggcc atggacacgg gactctacat ctgcaaggtg 360

gagctcatgt acccaccgcc atactacgag ggcataggca acggaaccca gatttatgta 420

attgatccag aaccgtgccc agattctgat caggagccca aatcttctga caaaactcac 480

acatccccac cgtccccagc acctgaactc ctgggggat cgtcagtctt cctcttcccc 540

ccaaaaccca aggacaccct catgatctcc cggacccctg aggtcacatg cgtggtggtg 600

gacgtgagcc acgaagaccc tgaggtcaag ttcaactggt acgtggacgg cgtggaggtg 660

cataatgcca agacaaagcc gcgggaggag cagtacaaca gcacgtaccg tgtggtcagc 720

gtcctcaccg tcctgcacca ggactggctg aatggcaagg agtacaagtg caaggtctcc 780

aacaaagccc tcccagcccc catcgagaaa accatctcca aagccaaagg gcagccccga 840

gaaccacagg tgtacaccct gcccccatcc cgggatgagc tgaccaagaa ccaggtcagc 900

ctgacctgcc tggtcaaagg cttctatccc agcgacatcg ccgtggagtg ggagagcaat 960

gggcagccgg agaacaacta caagaccacg cctcccgtgc tggactccga cggctccttc 1020

ttcctctaca gcaagctcac cgtggacaag agcaggtggc agcaggggaa cgtcttctca 1080

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ccgggtaaat ga 1152

<210> 14
<211> 383
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: L104EA29YIg

<400> 14

Met Gly Val Leu Leu Thr Gln Arg Thr Leu Leu Ser Leu Val Leu Ala
1 5 10 15

Leu Leu Phe Pro Ser Met Ala Ser Met Ala Met His Val Ala Gln Pro
20 25 30

Ala Val Val Leu Ala Ser Ser Arg Gly Ile Ala Ser Phe Val Cys Glu
35 40 45

Tyr Ala Ser Pro Gly Lys Tyr Thr Glu Val Arg Val Thr Val Leu Arg
50 55 60

Gln Ala Asp Ser Gln Val Thr Glu Val Cys Ala Ala Thr Tyr Met Met
65 70 75 80

Gly Asn Glu Leu Thr Phe Leu Asp Asp Ser Ile Cys Thr Gly Thr Ser
85 90 95

Ser Gly Asn Gln Val Asn Leu Thr Ile Gln Gly Leu Arg Ala Met Asp
100 105 110

Thr Gly Leu Tyr Ile Cys Lys Val Glu Leu Met Tyr Pro Pro Pro Tyr
115 120 125

Tyr Glu Gly Ile Gly Asn Gly Thr Gln Ile Tyr Val Ile Asp Pro Glu
130 135 140

Pro Cys Pro Asp Ser Asp Gln Glu Pro Lys Ser Ser Asp Lys Thr His
145 150 155 160

Thr Ser Pro Pro Ser Pro Ala Pro Glu Leu Leu Gly Gly Ser Ser Val
165 170 175

Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr
180 185 190

Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu
195 200 205

Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys
210 215 220

Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser
225 230 235 240

Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys
245 250 255

Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile
260 265 270

Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro
275 280 285

Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Thr Cys Leu
290 295 300

Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn
305 310 315 320

Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser
325 330 335

Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg

340 345 350

Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu
355 360 365

His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
370 375 380

<210> 15
<211> 1152
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: L104EA29LIg

<400> 15
atgggtgtac tgctcacaca gaggacgctg ctcagtctgg tccttgcact cctgtttcca 60

agcatggcga gcatggcaat gcacgtggcc cagcctgctg tggtactggc cagcagccga 120

ggcatcgcta gctttgtgtg tgagtatgca tctccaggca aattgactga ggtccgggtg 180

acagtgcttc ggcaggctga cagccaggtg actgaagtct gtgcggcaac ctacatgatg 240

gggaatgagt tgaccttcct agatgattcc atctgcacgg gcacctccag tggaaatcaa 300

gtgaacctca ctatccaagg actgagggcc atggacacgg gactctacat ctgcaaggtg 360

gagctcatgt acccaccgcc atactacgag ggcataggca acggaaccca gatttatgta 420

attgatccag aaccgtgccc agattctgat caggagccca aatcttctga caaaactcac 480

acatccccac cgtccccagc acctgaactc ctgggggat cgtcagtctt cctcttcccc 540

ccaaaaccca aggacaccct catgatctcc cggacccctg aggtcacatg cgtggtggtg 600

gacgtgagcc acgaagaccc tgaggtcaag ttcaactggt acgtggacgg cgtggaggtg 660

cataatgcca agacaaagcc gcgggaggag cagtacaaca gcacgtaccg tgtggtcagc 720

gtcctcaccg tcctgcacca ggactggctg aatggcaagg agtacaagtg caaggtctcc 780

aacaaagccc tcccagcccc catcgagaaa accatctcca aagccaaagg gcagccccga 840

gaaccacagg tgtacaccct gcccccatcc cgggatgagc tgaccaagaa ccaggtcagc 900

ctgacctgcc tggtcaaagg cttctatccc agcgacatcg ccgtggagtg ggagagcaat 960

gggcagccgg agaacaacta caagaccacg cctcccgtgc tggactccga cggctccttc 1020

ttcctctaca gcaagctcac cgtggacaag agcaggtggc agcaggggaa cgtcttctca 1080

tgctccgtga tgcatgaggc tctgcacaac cactacacgc agaagagcct ctccctgtct 1140

ccgggtaaat ga 1152

<210> 16
<211> 383
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: L104EA29LIg

<400> 16

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Met Gly Val Leu Leu Thr Gln Arg Thr Leu Leu Ser Leu Val Leu Ala
1               5                   10                  15

Leu Leu Phe Pro Ser Met Ala Ser Met Ala Met His Val Ala Gln Pro
            20                  25                  30

Ala Val Val Leu Ala Ser Ser Arg Gly Ile Ala Ser Phe Val Cys Glu
        35                  40                  45

Tyr Ala Ser Pro Gly Lys Leu Thr Glu Val Arg Val Thr Val Leu Arg
    50                  55                  60

Gln Ala Asp Ser Gln Val Thr Glu Val Cys Ala Ala Thr Tyr Met Met
65                  70                  75                  80

Gly Asn Glu Leu Thr Phe Leu Asp Asp Ser Ile Cys Thr Gly Thr Ser
                85                  90                  95

Ser Gly Asn Gln Val Asn Leu Thr Ile Gln Gly Leu Arg Ala Met Asp
            100                 105                 110

Thr Gly Leu Tyr Ile Cys Lys Val Glu Leu Met Tyr Pro Pro Pro Tyr
        115                 120                 125

Tyr Glu Gly Ile Gly Asn Gly Thr Gln Ile Tyr Val Ile Asp Pro Glu
    130                 135                 140

Pro Cys Pro Asp Ser Asp Gln Glu Pro Lys Ser Ser Asp Lys Thr His
145                 150                 155                 160

Thr Ser Pro Pro Ser Pro Ala Pro Glu Leu Leu Gly Gly Ser Ser Val
                165                 170                 175
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Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr
180 185 190

Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu
195 200 205

Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys
210 215 220

Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser
225 230 235 240

Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys
245 250 255

Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile
260 265 270

Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro
275 280 285

Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Thr Cys Leu
290 295 300

Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn
305 310 315 320

Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser
325 330 335

Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg
340 345 350

Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu
355 360 365

His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
370 375 380

<210> 17
<211> 1152
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: L104EA29TIg

<400> 17
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agcatggcga gcatggcaat gcacgtggcc cagcctgctg tggtactggc cagcagccga   120

ggcatcgcta gctttgtgtg tgagtatgca tctccaggca aaactactga ggtccgggtg   180

acagtgcttc ggcaggctga cagccaggtg actgaagtct gtgcggcaac ctacatgatg   240

gggaatgagt tgaccttcct agatgattcc atctgcacgg gcacctccag tggaaatcaa   300

gtgaacctca ctatccaagg actgagggcc atggacacgg gactctacat ctgcaaggtg   360

gagctcatgt acccaccgcc atactacgag ggcataggca acggaaccca gatttatgta   420

attgatccag aaccgtgccc agattctgat caggagccca aatcttctga caaaactcac   480

acatccccac cgtccccagc acctgaactc ctggggggat cgtcagtctt cctcttcccc   540

ccaaaaccca aggacaccct catgatctcc cggacccctg aggtcacatg cgtggtggtg   600

gacgtgagcc acgaagaccc tgaggtcaag ttcaactggt acgtggacgg cgtggaggtg   660

cataatgcca agacaaagcc gcgggaggag cagtacaaca gcacgtaccg tgtggtcagc   720

gtcctcaccg tcctgcacca ggactggctg aatggcaagg agtacaagtg caaggtctcc   780

aacaaagccc tcccagcccc catcgagaaa accatctcca aagccaaagg gcagccccga   840

gaaccacagg tgtacaccct gcccccatcc cgggatgagc tgaccaagaa ccaggtcagc   900

ctgacctgcc tggtcaaagg cttctatccc agcgacatcg ccgtggagtg ggagagcaat   960

gggcagccgg agaacaacta caagaccacg cctcccgtgc tggactccga cggctccttc 1020

ttcctctaca gcaagctcac cgtggacaag agcaggtggc agcaggggaa cgtcttctca 1080

tgctccgtga tgcatgaggc tctgcacaac cactacacgc agaagagcct ctccctgtct 1140

ccgggtaaat ga   1152

<210> 18
<211> 383
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: L104EA29TIg

<400> 18

Met Gly Val Leu Leu Thr Gln Arg Thr Leu Leu Ser Leu Val Leu Ala

1 5 10 15

Leu Leu Phe Pro Ser Met Ala Ser Met Ala Met His Val Ala Gln Pro
20 25 30

Ala Val Val Leu Ala Ser Ser Arg Gly Ile Ala Ser Phe Val Cys Glu
35 40 45

Tyr Ala Ser Pro Gly Lys Thr Thr Glu Val Arg Val Thr Val Leu Arg
50 55 60

Gln Ala Asp Ser Gln Val Thr Glu Val Cys Ala Ala Thr Tyr Met Met
65 70 75 80

Gly Asn Glu Leu Thr Phe Leu Asp Asp Ser Ile Cys Thr Gly Thr Ser
85 90 95

Ser Gly Asn Gln Val Asn Leu Thr Ile Gln Gly Leu Arg Ala Met Asp
100 105 110

Thr Gly Leu Tyr Ile Cys Lys Val Glu Leu Met Tyr Pro Pro Pro Tyr
115 120 125

Tyr Glu Gly Ile Gly Asn Gly Thr Gln Ile Tyr Val Ile Asp Pro Glu
130 135 140

Pro Cys Pro Asp Ser Asp Gln Glu Pro Lys Ser Ser Asp Lys Thr His
145 150 155 160

Thr Ser Pro Pro Ser Pro Ala Pro Glu Leu Leu Gly Gly Ser Ser Val
165 170 175

Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr
180 185 190

Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu
195 200 205

Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys
210 215 220

Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser
225 230 235 240

Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys
245 250 255

Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile
260 265 270

Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro
275 280 285

Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Thr Cys Leu
290 295 300

Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn
305 310 315 320

Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser
325 330 335

Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg
340 345 350

Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu
355 360 365

His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
370 375 380

<210> 19
<211> 1152
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: L104EA29WIg

<400> 19
atgggtgtac tgctcacaca gaggacgctg ctcagtctgg tccttgcact cctgtttcca 60

agcatggcga gcatggcaat gcacgtggcc cagcctgctg tggtactggc cagcagccga 120

ggcatcgcta gctttgtgtg tgagtatgca tctccaggca aatggactga ggtccgggtg 180

acagtgcttc ggcaggctga cagccaggtg actgaagtct gtgcggcaac ctacatgatg 240

gggaatgagt tgaccttcct agatgattcc atctgcacgg gcacctccag tggaaatcaa 300

gtgaacctca ctatccaagg actgagggcc atggacacgg gactctacat ctgcaaggtg 360

gagctcatgt acccaccgcc atactacgag ggcataggca acggaaccca gatttatgta 420

attgatccag aaccgtgccc agattctgat caggagccca aatcttctga caaaactcac 480

acatccccac cgtccccagc acctgaactc ctgggggat cgtcagtctt cctcttcccc 540

ccaaaaccca aggacaccct catgatctcc cggacccctg aggtcacatg cgtggtggtg 600

gacgtgagcc acgaagaccc tgaggtcaag ttcaactggt acgtggacgg cgtggaggtg 660

cataatgcca agacaaagcc gcgggaggag cagtacaaca gcacgtaccg tgtggtcagc 720

gtcctcaccg tcctgcacca ggactggctg aatggcaagg agtacaagtg caaggtctcc 780

aacaaagccc tcccagcccc catcgagaaa accatctcca aagccaaagg gcagccccga 840

gaaccacagg tgtacaccct gcccccatcc cgggatgagc tgaccaagaa ccaggtcagc 900

ctgacctgcc tggtcaaagg cttctatccc agcgacatcg ccgtggagtg ggagagcaat 960

gggcagccgg agaacaacta caagaccacg cctcccgtgc tggactccga cggctccttc 1020

ttcctctaca gcaagctcac cgtggacaag agcaggtggc agcaggggaa cgtcttctca 1080

tgctccgtga tgcatgaggc tctgcacaac cactacacgc agaagagcct ctccctgtct 1140

ccgggtaaat ga 1152

<210> 20
<211> 383
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: L104EA29WIg

<400> 20

Met Gly Val Leu Leu Thr Gln Arg Thr Leu Leu Ser Leu Val Leu Ala
1 5 10 15

Leu Leu Phe Pro Ser Met Ala Ser Met Ala Met His Val Ala Gln Pro
20 25 30

Ala Val Val Leu Ala Ser Ser Arg Gly Ile Ala Ser Phe Val Cys Glu
35 40 45

Tyr Ala Ser Pro Gly Lys Trp Thr Glu Val Arg Val Thr Val Leu Arg
50 55 60

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Gln Ala Asp Ser Gln Val Thr Glu Val Cys Ala Ala Thr Tyr Met Met
65                  70                  75                  80

Gly Asn Glu Leu Thr Phe Leu Asp Asp Ser Ile Cys Thr Gly Thr Ser
                85                  90                  95

Ser Gly Asn Gln Val Asn Leu Thr Ile Gln Gly Leu Arg Ala Met Asp
            100                 105                 110

Thr Gly Leu Tyr Ile Cys Lys Val Glu Leu Met Tyr Pro Pro Pro Tyr
        115                 120                 125

Tyr Glu Gly Ile Gly Asn Gly Thr Gln Ile Tyr Val Ile Asp Pro Glu
    130                 135                 140

Pro Cys Pro Asp Ser Asp Gln Glu Pro Lys Ser Ser Asp Lys Thr His
145                 150                 155                 160

Thr Ser Pro Pro Ser Pro Ala Pro Glu Leu Leu Gly Gly Ser Ser Val
                165                 170                 175

Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr
            180                 185                 190

Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu
        195                 200                 205

Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys
    210                 215                 220

Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser
225                 230                 235                 240

Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys
                245                 250                 255

Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile
            260                 265                 270

Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro
        275                 280                 285

Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Thr Cys Leu
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290 295 300

Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn
305 310 315 320

Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser
325 330 335

Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg
340 345 350

Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu
355 360 365

His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
370 375 380

<210> 21
<211> 65
<212> DNA
<213> Homo sapiens

<400> 21
ctcagtctgg tccttgcact cctgtttcca agcatggcga gcatggcaat gcacgtggcc 60

cagcc 65

<210> 22
<211> 33
<212> DNA
<213> Homo sapiens

<400> 22
tttgggctcc tgatcagaat ctgggcacgg ttg 33

<210> 23
<211> 72
<212> DNA
<213> Homo sapiens

<400> 23
ctagccactg aagcttcacc aatgggtgta ctgctcacac agaggacgct gctcagtctg 60

gtccttgcac tc 72

<210> 24
<211> 33
<212> DNA

<213> Homo sapiens

<400> 24
gcaatgcacg tggcccagcc tgctgtggta gtg   33

<210> 25
<211> 45
<212> DNA
<213> Homo sapiens

<400> 25
tgatgtaaca tgtctagatc aattgatggg aataaaataa ggctg   45

<210> 26
<211> 39
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Oncostatin M signal peptide f
       orward primer

<400> 26
ctagccactg aagcttcacc atgggtgtac tgctcacac   39

<210> 27
<211> 39
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Oncostatin M signal peptide r
       everse primer

<400> 27
tggcatgggc tcctgatcag gcttagaagg tccgggaaa   39

<210> 28
<211> 39
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Oncostatin M signal peptide r
       everse primer

<400> 28
tttgggctcc tgatcaggaa aatgctcttg cttggttgt   39

<210> 29
<211> 84
<212> DNA

<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Human IgCgamma1 forward prime
r

<400> 29
aagcaagagc atttcctga tcaggagccc aaatcttctg acaaaactca cacatcccca 60

ccgtccccag cacctgaact cctg 84

<210> 30
<211> 41
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Human IgCgamma1 reverse prime
r

<400> 30
cttcgaccag tctagaagca tcctcgtgcg accgcgagag c 41

<210> 31
<211> 47
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: CD5Ig forward primer

<400> 31
cattgcacag tcaagcttcc atgcccatgg gttctctggc caccttg 47

<210> 32
<211> 39
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: CD5Ig reverse primer

<400> 32
atccacagtg cagtgatcat ttggatcctg gcatgtgac 39

<210> 33
<211> 18
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: CDM8 forward primer

<400> 33
aatacgactc actatagg 18

<210> 34
<211> 18
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: CDM8 reverse primer

<400> 34
caccacactg tattaacc 18

<210> 35
<211> 6
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: CTLA4/CD28

<400> 35

Met Tyr Pro Pro Pro Tyr
1 5

<210> 36
<211> 6
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: CTLA4Ig mutant fusion protein

<400> 36

Ala Tyr Pro Pro Pro Tyr
1 5

<210> 37
<211> 6
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: CTLA4Ig mutant fusion protein

<400> 37

Met Ala Pro Pro Pro Tyr
1 5

<210> 38
<211> 6
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: CTLA4Ig mutant fusion protein

<400> 38

Met Tyr Ala Pro Pro Tyr
1   5

<210> 39
<211> 6
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: CTLA4Ig mutant fusion protein

<400> 39

Met Tyr Pro Ala Pro Tyr
1   5

<210> 40
<211> 6
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: CTLA4/CD28Ig mutant fusion pr
otein

<400> 40

Met Tyr Pro Pro Ala Tyr
1   5

<210> 41
<211> 6
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: CTLA4/CD28Ig mutant fusion pr
otein

<400> 41

Met Tyr Pro Pro Pro Ala
1   5

<210> 42
<211> 6
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: CTLA4Ig mutant fusion protein

<400> 42

Ala Ala Pro Pro Pro Tyr
1 5

<210> 43
<211> 68
<212> DNA
<213> Artificial Sequence

<220>
<223> k is either c, g or t

<220>
<221> misc_feature
<222> (28)..(29)
<223> n is either a, c, g or t

<220>
<221> misc_feature
<222> (30)..(30)
<223> k is either c, g or t

<220>
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<222> (31)..(32)
<223> n is either a, c, g or t

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<222> (33)..(33)
<223> k is either c, g or t

<220>
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<222> (34)..(35)
<223> n is either a, c, g or t

<220>
<221> misc_feature
<222> (36)..(36)

<223> k is either c, g or t

<220>
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<222> (37)..(38)
<223> n is either a, c, g or t

<220>
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<222> (39)..(39)
<223> k is either c, g or t

<220>
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<223> n is either a, c, g or t

<220>
<221> misc_feature
<222> (42)..(42)
<223> k is either c, g or t

<220>
<221> misc_feature
<222> (43)..(44)
<223> n is either a, c, g or t

<220>
<221> misc_feature
<222> (45)..(45)
<223> k is either c, g or t

<220>
<221> misc_feature
<222> (46)..(47)
<223> n is either a, c, g or t

<220>
<221> misc_feature
<222> (48)..(48)
<223> k is either c, g or t

<220>
<221> misc_feature
<222> (49)..(50)
<223> n is either a, c, g or t

<220>
<221> misc_feature
<222> (51)..(51)
<223> k is either c, g or t

<400> 43
cgaggcatcg ctagctttgt gtgtgagnnk nnknnknnkn nknnknnknn kgaggtccgg 60

gtgacagt 68

<210> 44
<211> 59
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Phage display reverse primer

<400> 44
ggttgccgca cagacttcgg tcacctggct gtcagcctgc cgaagcactg tcacccgga 59

<210> 45
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: CTLA4 mutant

<400> 45

Phe Glu Pro Lys Arg Gly Val Gln
1   5

<210> 46
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: CTLA4 mutant

<400> 46

Trp Asp Gln Tyr Thr Gly Tyr Gly
1   5

<210> 47
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: CTLA4 mutant

<400> 47

Trp Asp Ala Tyr Arg Asn Gln Gln
1 5

<210> 48
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: CTLA4 mutant

<400> 48

Tyr Asp His Pro Tyr Asp Gly Gln
1 5

<210> 49
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: CTLA4 mutant

<400> 49

Trp Asp Gln His Val Ser Arg Arg
1 5

<210> 50
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: CTLA4

<400> 50

Tyr Ala Ser Pro Gly Lys Ala Thr
1 5